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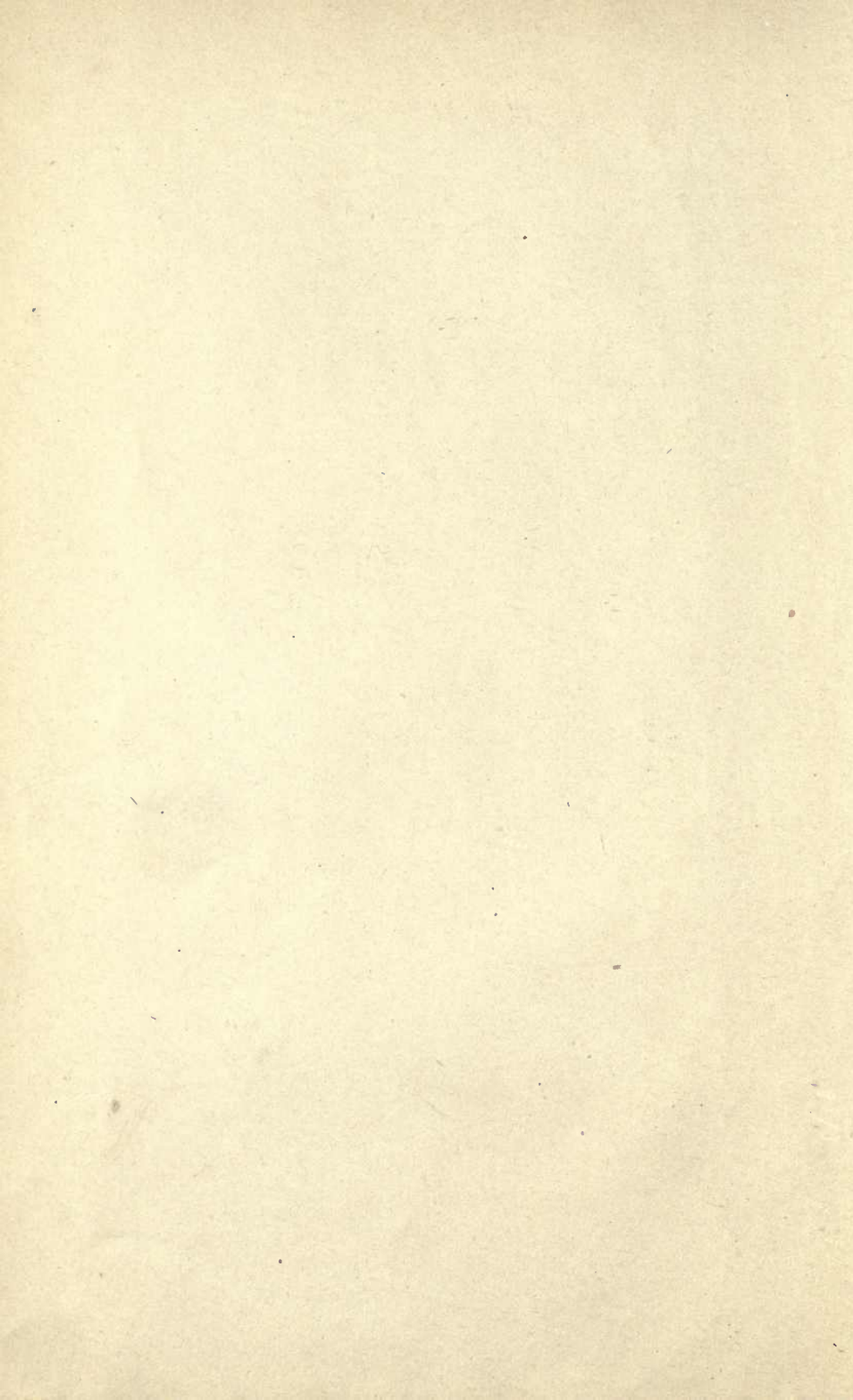


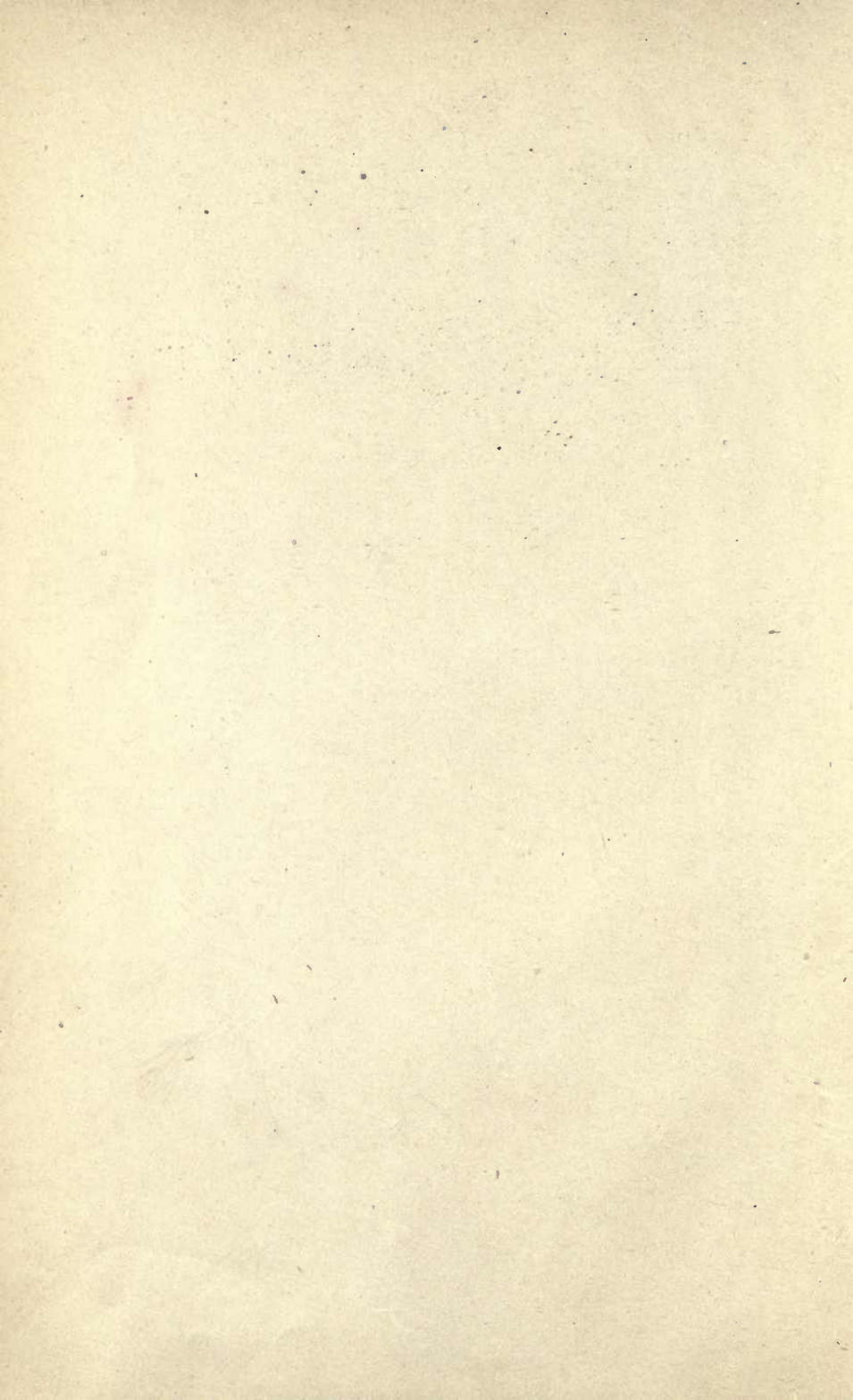
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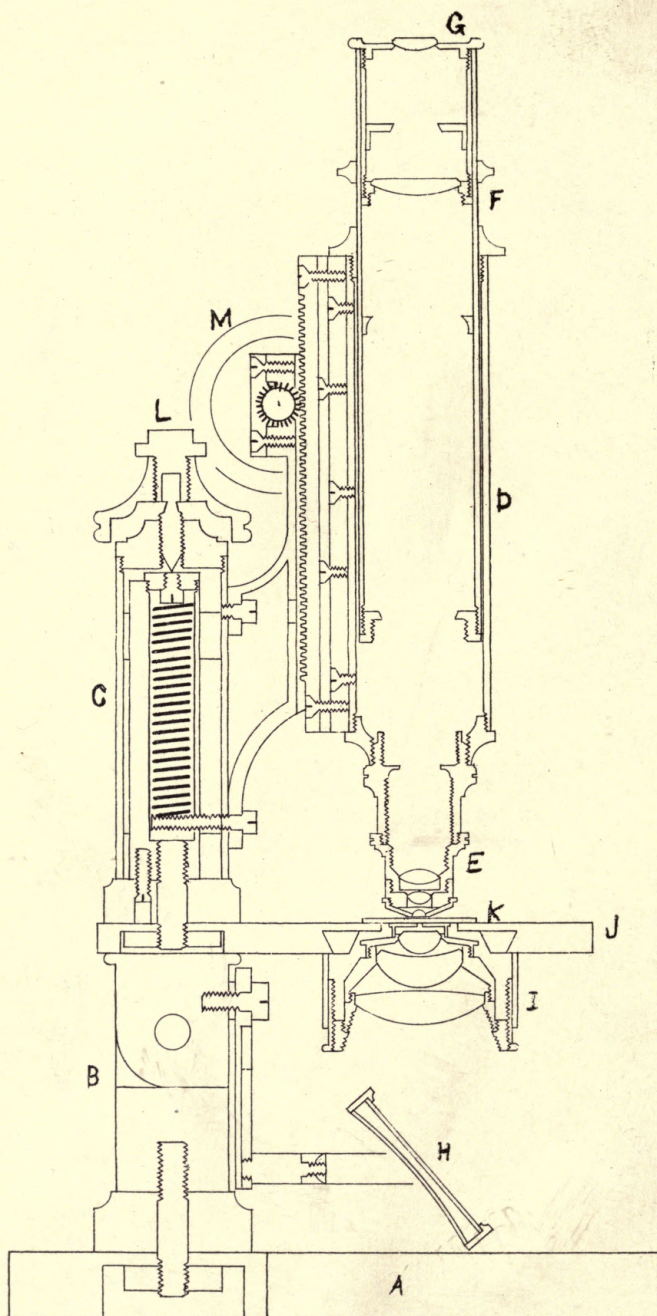
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DIRECTIONS

FOR WORK IN

Histological Laboratory

FOR THE USE OF

MEDICAL CLASSES

IN THE

UNIVERSITY OF MICHIGAN.

G. CARL HUBER, M. D.,

ASSISTANT PROFESSOR OF HISTOLOGY AND EMBRYOLOGY.

GEORGE WAHR,
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PREFACE.

The following pages have been prepared with the hope that they may be a guide and a help to the students doing work in the Histological Laboratory. In the very brief description that is given of the tissues to be studied I have born in mind that the students entering on this work, will have had, a course of lectures on Histology. The aim of the notes is therefore not to *supplant*, but rather to *supplement* the text-books on this subject.

Drawings are to be made of nearly all the preparations to be examined. There is no better way for the student to obtain a correct and a lasting impression of the tissues to be studied than by carefully sketching what he sees under the microscope. The drawings are to be made in the laboratory. A few methods for hardening, macerating, embedding and staining tissues are given; such as have proved themselves to be most reliable have been selected. Anyone familiar with the methods here given can without difficulty employ any he may find recorded in the works on microscopical technic.

I am indebted to the Bausch & Lomb Optical Company for sectional diagram of their Model microscope; to Mr. S. P. Budgett for the drawings of the two wood cuts. I also wish to acknowledge the help received in the arrangement of lessons, from Schäffer's Essentials of Histology.

G. C. H.

M347970

SUPPLIES.

The student before entering on the work should supply himself with the following outfit :

Six dozen slides; $\frac{1}{2}$ ounce No. 2 $\frac{3}{4}$ -inch square cover glasses and 2 dozen No. 2 $\frac{3}{4}$ -inch circles; a slide box; a sharp razor, flat on one side; a fine pair of scissors; 2 teasing needles; 3 solid watch crystals; 1 section lifter; 1 Camels hair brush; a hard and a soft drawing pencil; 1 Canada balsam tube; and 1 pair of cover-glass forceps.

BOOKS OF REFERENCE.

- Toldt*.—Lehrbuch der Gewebelehre.
Behrens, Kossel, Schiefferdecker.—Die Gewebe des Menschlichen Körpers.
Kölliker.—Handbuch der Gewebelehre des Menschen.
Schwalbe.—Lehrbuch der Anatomie der Sinnesorgane.
Cadiat.—Taité D Anatomie Générale.
Klein and Noble Smith.—Atlas of Histology.
Quain.—Elements of Anatomy. Vol. I. Part II. General Anatomy or Histology, by Prof. Schäffer.
Satterthwaite.—Manual of Histology.
Orth.—Normale Histologie.
Stöhr.—Lehrbuch der Histologie.
Microscopical Technic.—
Lee.—The Microtometist's Vade-Mecum.
Whitman.—Methods of Microscopical Anatomy and Embryology.
Behrens.—Tabellen zum Gebrauch bei Mikroskopischen Arbeiten.
Ranvier.—Traité technique d'histologie.
Boehm and Oppel.—Taschenbuch der Mikroskopischen Technik.
Friedlander-Eberth.—Mikroskopische Technik zum Gebrauch bei Medicinischen und Pathologisch-anatomischen Untersuchungen.
Heneage Gibbs.—Practical Histology and Pathology.
Kahlden.—Technik der Histologischen Untersuchung Pathologisch-anatomischer Präparate.
Rawitz.—Leitfaden der Histologischen Untersuchungen.
Behrens, Kossel, Schiefferdecker.—Vol. I.

LESSON I.

CELL AND CELL DIVISION.

(a) PLANT CELLS.

From one of the layers of an onion remove a small strip of the film which is found on its inner surface. Spread out this thin membrane on a slide in a drop of water, and cover with a "cover glass." In studying it use first the low power, and employ one of the smaller openings in the diaphragm. Observe the large cells, oblong or nearly square in shape, each bounded by a distinct cell membrane. Notice the round or oval nucleus, usually seen at one end, or near one of the sides of the cell.

Carefully elevate the cover glass from one edge, and add a drop of "Lugol's solution," to the water; observe the staining. Make a sketch of a number of the cells as seen under the high power.

(b) KARYOKINESIS IN PLANT CELLS.

The young and growing "tips" of an onion were hardened in Fleming's solution, embedded in celloidin, sectioned, and the sections were stained in Boehmer's hæmatoxylin, they were then dehydrated in alcohol, and are now in oil of bergamot. To make a "permanent mount," place a section on a slide, remove excess of oil by means of a small piece of filter paper, add a drop of Canada balsam, and cover with a "cover slip." Sections were made longitudinally through the "tip," and under low power the parallel rows of cells will be seen. Even with this power it will be noticed that some of the nuclei stain much more deeply than others, examine these with

the high power and one of the stages of cell division (Karyokinesis) will be recognized. Observe the difference in the appearance of a "resting nucleus" and one that is in the earlier stages of cell division; in the latter the chromosoma can be clearly determined, while in the former a "chromatic network" is presented. Search for cells in which the chromosoma are arranged in the form of a *monaster*, and also such as show the *diaster stage*. You may be able to make out the achromatic spindle.

Make a sketch of the different stages of mitosis presented in your preparation, as seen under high power.

(a) **UNICELLULAR ORGANISMS.**

In the faecal matter found in the rectum of a frog, it is not uncommon to find, as parasites, unicellular organisms belonging to the ciliata or flagellata. Place a small portion of the faecal matter on a slide and add a drop of normal salt solution (NaCl 0.6% in distilled water) cover with a slip. Under the microscope these little animals will be seen darting through the field by means of their ciliary movement. Place a small drop of a one per cent. osmic acid solution to one side of the cover glass, (near enough to touch the edge) and with a piece of filter paper, held to the opposite edge of the slip, carefully remove, by absorption, some of the fluid under the cover; in this way some of the osmic acid will be drawn under, and will in a few moments fix the living cells suspended in the fluid. Note their size, shape and the structure of protoplasm and nucleus.

DRAWINGS FOR LESSON I.

DRAWINGS FOR LESSON I.

LESSON II.

CELL AND CELL DIVISION (CONTINUED).

(a) **TYPICAL ANIMAL CELLS.**

A small portion taken from the ovary of a young frog is to be teased in normal salt solution, cover with a "cover glass," and examine with the low power. Observe the large spherical cells, with granular protoplasm and a nucleus which is only indistinctly discernible. Elevate the edge of the cover glass, and add a few drops of a one per cent. solution of acetic acid; this "clears" the protoplasm, and now a more careful study of the nucleus can be made.

Make a sketch of several of the ova as seen under the low power, after the acetic acid has been added.

(b) **KARYOKINESIS IN ANIMAL CELLS.**

Fleming has long ago shown that the salamander testis is one of the most useful tissues for showing Karyokinesis in animal cells. The testes were hardened in "Fleming's solution," embedded in paraffin, sections were cut and these were fixed to a cover glass by means of an "albumen fixative," the paraffin was removed, they were stained in safranin, dehydrated, cleared, and are now in xylol. Bring a slide on which has been placed a small drop of Canada balsam, and the preparation will be mounted for you at the "distributing table." In this lesson only the structure of the cells and the different stages of Karyokinesis are to be studied. Sections need to be examined with a high power. The great majority of the cells will be "resting cells," showing a nucleus possessing a chromatic, intranuclear network and a nucleolus, a protoplasm with an intracellular network.

By moving the sections about, cells fixed in the process of division will be found. Make a sketch of a cell, not in the process of division, and indicate in your drawing, as clearly as you can, the finer structure of the protoplasm and nucleus. Reproduce the different stages of Karyokinesis your specimen shows.

(c) **DEMONSTRATION.**

Under a one-twelfth oil immersion, will be demonstrated a section taken from a salamander testis, fixed and stained to show *accessory nucleus* (Nebenkern) in a resting cell. Also cells fixed in process of division, showing *chromosoma*, *microsoma*, and the different parts of the *achromatic spindle*.

DRAWINGS FOR LESSON II.

DRAWINGS FOR LESSON II.

LESSON III.

MAMMALIAN BLOOD.

(a) FRESH HUMAN BLOOD.

Obtain a drop of blood by pricking a carefully cleaned finger with a steel pen, one of the prongs of which has been broken off, quickly mount the drop on a slide, and examine with the high power. Observe that most of the red cells are arranged in rouleaux, and between these now and then a white corpuscle is seen. If your drop was small, some of the red corpuscles found in the peripheral part of your preparation will appear crenated.

(b) FRESH HUMAN BLOOD WITH NORMAL SALT.

Obtain a very small drop of blood as above directed, mix it on the slide with a drop of normal salt solution, and cover. The smaller number of corpuscles will allow of a more careful study of their size and shape. Observe that the red appear as biconcave circular discs, and are a little smaller than the majority of the white. Some of the red will soon become crenated.

Make a drawing of a number of the red cells as seen on "the flat," and a few seen on the edge (profile).

(c) FIBRIN.

Place a large drop of blood on the slide, cover with a "slip" and allow it to clot. When clotted, wash gently with a current of water, (by placing a few drops on one side of the cover and touching the opposite edge with a piece of filter paper,) so that some of the red blood cells may be removed.

Examine now with the high power, and see whether the delicate, glistening strands of fibrin are noticeable in

the field. This preparation may now be stained by causing a 0.5% solution of Methylenblue to flow under the cover glass. The fibrin filaments and the white blood cells will take on the blue color.

(d) **MAMMALIAN BLOOD FIXED WITH HAYEM'S SOLUTION.**

Blood from the carotid artery of a cat was allowed to flow into a quantity of Hayem's solution, the fixed elements sank to the bottom, and in 24 hours the supernatant fluid was decanted, the corpuscles were then washed in distilled water, and after washing mixed with a small quantity of gum glycerin. A small drop of the mixture is to be taken on a slide, and covered; this will give you a permanent mount. In it the size and shape of the red corpuscles, as well as the structure of the white blood cells, can be studied.

(e) **DEMONSTRATION.**

In a blood preparation, fixed and stained according to Ehrlich's method, the "blood plates" will be demonstrated under an one-twelfth inch oil immersion.

DRAWINGS FOR LESSON III.

DRAWINGS FOR LESSON III.

LESSON IV.

AVIAN AND AMPHIBIAN BLOOD.

(a) PIGEON BLOOD.

One of the toes of a pigeon has been amputated, from the flowing blood allow a drop to fall on the slide, mix with a drop of normal salt solution and cover with a slip. Examine under high power. Observe that the red corpuscles are oval, somewhat flattened and nucleated; note the relative size of red and white cells. Cause a drop of a one per cent. solution of acetic acid to flow under the cover, and you will notice that the nuclei in the red and white cells can be more clearly seen.

Make a sketch of several red and white corpuscles, as they present themselves to you after the acid has been added.

(b) PIGEON BLOOD HARDENED IN HAYEM'S SOLUTION.

A pigeon was bled into a large quantity of Hayem's solution, the blood was hardened and washed as described in yesterday's lesson, and is now in gum glycerin. A small drop is to be mounted on a slide, this will serve as a "permanent mount," and is to be compared with the preparation of blood already made.

(c) AMPHIBIAN BLOOD.

A drop of blood taken from the opened heart of a frog is placed on a slide and mixed with a drop of normal salt solution, cover with a "slip." The red corpuscles are oval, somewhat flattened and nucleated cells, much larger than the white. Observe also, that when the red are viewed in profile, they show a slight convexity in the centre. Add a drop of acetic acid (one per cent. solution),

and the nuclei of the red and white corpuscles will stand out clearly, in those parts of the field where the acid has come in contact with them.

Reproduce some of the red as seen on “the flat,” and others seen in profile, also a number of the white.

(d) **AMPHIBIAN BLOOD FIXED WITH HAYEM'S SOLUTION.**

Blood cells of a frog were hardened in Hayem's solution, and are now in gum glycerin, mount a drop of the mixture. Compare the cat's and the pigeon's blood with this preparation of frog's blood; the two latter show nucleated red cells, the former not. Note the difference in shape and size.

DRAWINGS FOR LESSON IV.

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DRAWINGS FOR LESSON IV.

LESSON V.

WHITE BLOOD AND LYMPH-CORPUSCLES AND THEIR AMOEBOID MOVEMENT.

(a) AMOEBOID MOVEMENT IN WHITE BLOOD CELLS OF A FROG.

A small quantity of blood obtained from the heart of a frog, is mixed with a drop of normal salt solution and covered with a slip, to prevent evaporation while examining, the following steps are taken: The cover glass is fixed by means of two or three drops of melted paraffin, so placed, that after congealing, one half of the drop will rest on the slide, the other on the edge of the cover; after this step has been taken, the sealing is completed by means of a camels-hair brush, which has been dipped in vaseline, and drawn about the edge of the cover. In examining this preparation be very careful not to bring the end of the objective in contact with the vaseline on the slide. Search the specimen until you find a white blood cell of somewhat irregular shape, place it in the centre of the field and make a drawing of it as it presents itself to you at this time; at intervals of one or two minutes, carefully repeat the sketching until about ten have been made, you will no doubt see that the cell in question has changed its shape a number of times, pseudopodia may have been thrown out and again withdrawn, etc.

(b) AMOEBOID MOVEMENT IN WHITE BLOOD CELLS OF CATS.

The animal from which this blood is to be taken, was severely bled 24 hours previously, a drop is to be obtained from a small incision in the ear, the manner of preparing and studying this preparation is the same as that described in the preceding paragraph of this lesson.

(c) **LYMPH CORPUSCLES.**

Scrape the cut surface of a fresh lymphatic gland with a scalpel, and mix the "scrapings" on the slide with a drop of normal salt solution. Cover and examine under high power, employing one of the smaller openings in the diaphragm, cells of different sizes will be seen; observe the structure of protoplasm and nucleus in the different forms presented. Cause a drop of a 1% solution of acetic acid to flow under the cover; in a few moments the nuclei will present themselves more clearly.

(d) **LYMPH CORPUSCLES FIXED AND STAINED WITH METHYLEN BLUE AND EOSIN.**

A freshly cut surface of a lymphatic gland was scraped, and the scraping spread on a cover glass; fixed by exposing it to a heat of 110°C, for 20 minutes, stained in methylen blue and eosin. Preparation will be completed for you at the "distributing table." On examining with the high power the following varieties of cells will be seen in the preparation:

(1) *Small lymphocytes*—in size about as large or a little larger than a red blood-cell, with a comparatively large nucleus, which takes the stain freely, and only a narrow border of protoplasm. The majority of the cells will belong to this order.

(2) *Large mononuclear white blood cells*—possessing a much larger border of protoplasm and a nucleus that stains but faintly; only a few will be found.

(3) *Ehrlich's transitional form*—with horse-shoe shaped nucleus.

(4) *Polynuclear white blood cells*—with a lobulated nucleus, staining very deeply.

Make a sketch of one cell from each of the above forms.

(e) **DEMONSTRATION.**

Demonstration of white blood cells which were stained, according to Ehrlich's methods. Given to show Eosinophile, Neutrophile, and Basophile cells.

DRAWINGS FOR LESSON V.

DRAWINGS FOR LESSON V.

LESSON VI.

EPITHELIUM.

(a) PAVEMENT EPITHELIUM, ISOLATED CELLS.

Scrape the inside of the cheek with the edge of a slide or a clean knife, mount the scrapings in a drop of saliva and cover. Study under high power and make use of one of the smaller openings in the diaphragm. Observe the large, flat epithelial cells. Many are single, others are found in groups. Note the round or oval nucleus.

You will also see the "salivary corpuscle," (leucocytes which have wandered through the oral epithelium, and are swollen by the imbibition of water,) cause a drop of methylen blue to flow under the cover, and the staining will bring to prominence the parts above described. Reproduce several pavement cells and a salivary corpuscle, as seen under high power.

(b) CUTANEOUS EPITHELIUM, FROM FROGS EPIDERMIS.

If a frog is imprisoned for some time in a jar containing a small quantity of water, it will be noticed that portions of the epidermis are from time to time shed and found floating in the water. These thin membranes were washed, stained in Bøhmer's hæmatoxylin, dehydrated in alcohol, and are now in oil of cloves, to complete the "mount," place the tissue on slide, remove excess of oil, add a drop of Canada balsam, and cover with a "slip." This preparation will give you a surface view of a stratified pavement epithelium.

(c) CROSS SECTION OF STRATIFIED EPITHELIUM.

A small portion of the mucous membrane lining the soft palate of a dog was hardened in a saturated watery solution of mercuric bichloride, stained in carmin, embedded in paraffin and sectioned.

The method of mounting paraffin sections is the following: Come to the distributing table with a slide on

which a thin layer of the "albumen fixative" has been spread, (place a *small* drop of the fixative on the centre of a clean slide, spread it out as thin as you can with a clean glass rod, then with a dry and clean finger wipe away all excess,) a section will then be fixed to the slide; now place slide on the water bath, and allow it to remain there until the paraffin begins to melt, quickly remove the melted paraffin, by covering the section with a few drops of oil of turpentine, (paraffin is soluble in oil of turpentine,) remove excess of oil, and add a drop of Canada balsam. Care should be taken not to *over-heat* the preparation, while the paraffin is being melted, nor to allow it to *dry* after the oil of turpentine has been removed.

Observe the several layers of epithelial cells, and their change in shape as they are traced outwards. In the lowest, the cells are columnar, in the following polygonal, while in the outermost layer, they are flattened.

Notice the change in the shape and structure of the nuclei, belonging to the cells of the different layers. Make a sketch as seen under high power.

(d) **ISOLATED COLUMNAR CELLS.**

The intestinal canal of a frog was dissociated in 33% alcohol for 18 hours, fixed in osmic acid, washed in water. A small portion of the epithelial lining is to be teased in gum glycerin, and mounted. Many isolated cells will be seen in the field, (use high power and one of the smaller openings in the diaphragm,) observe their shape, position of oval nucleus, and their striated border.

(e) **GOBLET CELLS.**

The mucous layer of the large intestine of a cat was macerated in 33% alcohol, and fixed in osmic acid (as above). Place a small portion of the tissue in a drop of methylen blue, and allow to stain for five minutes, wash in water, tease stained tissue in gum glycerin, and mount. You will notice columnar cells, also many in which the free, the inner portion of the cell appears distended, (by means of a mucigen which has formed there) these are the "goblet cells." Sketch several columnar, and two goblet cells as seen under the high power.

DRAWINGS FOR LESSON VI.

DRAWINGS FOR LESSON VI.

LESSON VII.

EPITHELIUM AND ENDOTHELIUM.

(a) **ISOLATED CELLS OF CILIATED EPITHELIUM.**

The mucous membrane of the trachea of a calf was placed in 33% alcohol for 12 hours, then into a $\frac{1}{2}\%$ sol. of osmic acid for 24 hours, stained in methylen blue and is to be teased in gum glycerin and mounted. Observe that most of the cells are columnar in shape, possessing an oval nucleus. Notice particularly the cluster of fine, short hairs adhering to the free end of the cell. Some of the cells may be distended with mucigen (goblet cells), if so no ciliary border will be seen. Sketch several as seen under the high power.

(b) **CROSS SECTION OF STRATIFIED COLUMNAR, CILIATED EPITHELIUM.**

The mucous membrane lining the palate of a frog, was hardened in "Fleming's solution," stained in alum carmin, embedded in paraffin and cut. Steps for mounting sections cut in paraffin were fully given in paragraph (c) of the last lesson. This epithelium is composed of three layers of cells, the cells of the outer layer being ciliated.

(c) **DEMONSTRATION OF CILIARY MOVEMENT.**

A small portion is removed from the pharynx of a frog and mounted in normal salt solution, the cover glass should be supported by means of a thick hair.

(d) **TRANSITIONAL EPITHELIUM.**

The bladder of a dog was injected with 33% sol. of alcohol, at the end of four hours it was cut into small pieces and these were allowed to remain in the above "dissociating fluid" for 12 hours, were then fixed in osmic acid, stained

in methylen blue and are to be teased and mounted in gum glycerin.

Observe the different forms of the cells presented; large, somewhat flattened cells, (depending on the degree of distention) often showing two nuclei, these belong to the inner stratum; the cells found in the next two layers are short columnar or "pear-shaped" and often quite irregular. Make a sketch of a number of them as seen under high power.

(c) **ENDOTHELIAL CELLS.**

A 1% sol. of silver nitrate was injected into the peritoneal cavity of a frog, after 15 minutes the intestines with the mesentery were removed, the latter, while fixed in an extended position, was exposed to sun light until the silver was reduced. Mount in gum glycerin. Observe that the silver is deposited in the intercellular cement, a delicate black line surrounding each cell, (the protoplasm and nucleus often take a light brown color).

Make a sketch of a number of these cells as seen under high power.

DRAWINGS FOR LESSON VII.

DRAWINGS FOR LESSON VII.

LESEON VIII.

CONNECTIVE TISSUE.

(a) FIBRILS OF WHITE FIBROUS CONNECTIVE TISSUE.

A short piece of a tendon was subjected to the digestive action of a glycerin extract of the pancreas, at a temperature of 35°C , (Ewald and Kühne). A small portion is to be teased in normal salt solution. If the teasing has been thoroughly done, many isolated elementary fibrils will be seen in the field. Observe that they do not anastomose, are often somewhat wavy. Cause a 1% solution of acetic acid to flow under the cover glass, and it will be seen that the fibrils swell up and become homogeneous.

(b) FIBRILS OF YELLOW ELASTIC CONNECTIVE TISSUE.

Tease a small portion of the elastic tissue, taken from the ligament nuchæ of an ox, in normal salt solution. Observe the fibrils of yellow elastic tissue, they are highly refractive, branch and anastomose, and the broken ends "curl back." Cause a few drops of 1% sol. of acetic acid to flow under the cover glass, and notice that the yellow elastic fibrils are not affected by it.

(c) AREOLAR CONNECTIVE TISSUE.

A small portion taken from the subcutaneous tissue of a young cat is placed on a dry slide, the edges are drawn out, and made to adhere to the slide by allowing them to dry, while the center is kept moist by breathing upon it. In this way a thin film may be obtained. Place a drop of normal salt on a cover glass and invert it over the center of the film.

In examining use the high power, and employ one of the smaller openings in the diaphragm. Observe the bun-

dles of white fibrous connective tissue, composed of the elementary fibrils, crossing the field in different directions; between which fibres of yellow elastic tissue will be seen, having a distinct outline, giving and receiving branches, and often possessing a straight course. Look for the connective tissue cells.

Carefully elevate the cover glass and add a drop of a 1% sol. acetic acid. In a few moments the bundles of white fibrous tissue will swell and appear homogeneous, while the unaffected yellow elastic fibers will stand out boldly; the nuclei of the fixed connective tissue cells can now be more clearly seen. Make a sketch of a bundle of white fibrous tissue, some yellow elastic fibers, and a number of the connective tissue cells.

(d) **TENDON.**

Tendons taken from the tail of a mouse were suspended in alum carmin for 24 hours. They are to be teased in gum glycerin. The tendon fasciculi are somewhat swollen, between them rows of tendon cells, which have taken the red color will be distinctly seen, they are oblong or square in shape, with the nucleus usually at one end.

Make sketch of two fasciculi with the intervening cells.

(e) **CROSS-SECTION OF TENDON.**

A tendon was hardened in chromic acid, cut, and stained in acid fuchsin. Sections are in oil of bergamot, and are to be mounted in Canada balsam. Examine with the low power. Observe that the tendon is surrounded by a connective tissue sheath composed of bundles of fibrous tissue, the majority of which run transversely; from this septa pass in, and divide the tendon into larger and smaller bundles, these are composed of tendon fasciculi, their cut ends being presented to you. Between the fasciculi the tendon corpuscles will be seen.

DRAWINGS FOR LESSON VIII.

DRAWINGS FOR LESSON VIII.

LESSON IX.

CONNECTIVE TISSUE. (CONTINUED.)

(a) **CROSS-SECTION OF LIGAMENT NUCHÆ.**

A small piece of the ligament nuchæ of an ox was allowed to dry, fine shavings are to be cut from one end, by means of a sharp scalpel. Place the section on a slide in a few drops of water, stain for five minutes in acid fuchsin, wash in water and mount in gum glycerin. Observe the cut end of the elastic fibres, arranged in smaller and larger bundles.

Make a sketch of a small portion as seen under high power.

(b) **EMBRYONIC CONNECTIVE TISSUE.**

Umbilical cord of human foetus was hardened in Müller's solution, stained in alum carmin, embedded in paraffin, and cross-sectioned. Fix the sections to the slide, remove the paraffin. On observing the preparation the umbilical vessels will be seen in cross-section, occupying the more central part of the section, in this lesson however the connective tissue is to receive special attention.

Observe the branched connective tissue cells, sending out long processes. The cells are separated by a large amount of a granular, intercellular ground substance, in which fine connective tissue fibrils, as well as the highly refractive elastic fibre, may be seen. Make a sketch as seen under high power.

(c) **FAT CELLS.**

A portion of the mesentery of a young rabbit is spread out on a slide, in a drop of normal salt solution. Examine

under low power, and notice the large, nearly spherical cells, collected into small groups, the lobules, in some of the cells a flattened nucleus may be seen, it having been pressed to one side. Cause a drop of osmic acid to flow under the slide and notice how the fat globules in the cells are blackened.

Make a sketch as seen under low power.

(d) **SECTION OF ADIPOSE TISSUE.**

Subcutaneous tissue, (from the sole of foot) was hardened in 10% nitric acid, washed, embedded in celloidin and sectioned. Sections were stained in Bøehmer's hæmatoxylin and eosin, dehydrated in alcohol and are now in oil of bergamot. Place section on the slide, remove excess of oil, and mount in Canada balsam. On studying this preparation you will find groups of fat cells, separated by bundles of connective tissue, this having been stained in the eosin. All nuclei are blue having taken the hæmatoxylin.

Sketch a few of the fat cells as seen under the high power.

DRAWINGS FOR LESSON IX.

DRAWINGS FOR LESSON IX.

LESSON X.

CARTILAGE.

(a) **HYALIN CARTILAGE.**

Remove by means of a sharp razor a thin section from the articular surface of a frog's femur, and mount in normal salt solution. Examine under high power, using one of the smaller openings in the diaphragm.

Observe the round or oval cartilage cells each surrounded by a capsule, and possessing a large spherical or oval nucleus. The cells are often found in groups of 2-4 or more, and are in small spaces (*the lacunæ*) in the homogeneous, intercellular ground substance, *the matrix*.

(b) **HYALIN CARTILAGE. (STAINED.)**

One of the ring cartilages, taken from the trachea of a young dog, was hardened in picric acid (saturated watery solution) embedded in celloidin and cross-sectioned. Sections were stained in hæmotoxylin and eosin, and are now in oil of bergamot, mount in Canada balsam. Note the perichondrium about the hyalin cartilage.

Observe the structure of the cartilage cells, and make a sketch as seen under high power, including in your drawing a small segment of the perichondrium and a portion of the adjoining hyalin cartilage.

(c) **ELASTIC FIBRO-CARTILAGE.**

The elastic cartilage was taken from a calf's ear, hardened in a saturated, watery solution of picric acid, embedded in celloidin, sectioned and sections were stained in picro-lithion-carmin, they are now in oil of bergamot, mount in Canada balsam.

Observe that in place of the homogeneous intercellular ground substance, as seen in hyalin cartilage, you have one that is permeated with a well defined network of yellow elastic fibres.

Make a drawing as seen under high power.

(d) **WHITE FIBRO-CARTILAGE.**

An intervertebral disc from a calf was hardened in a saturated watery solution of picric acid, embedded in celloidin, sectioned and the sections were stained in hæmatoxylin and eosin; they are now in oil of bergamot, mount in balsam.

In fibro-cartilage the intercellular ground substance is composed largely of bundles of white fibrous tissue, between these are found the cartilage cells, enclosed in their capsule, and often surrounded by a thin layer of a homogeneous, hyalin matrix.

Make a sketch as seen under the high power.

(e) **DEMONSTRATION OF THE LYMPH-CANALICULAR SYSTEM IN HYALIN CARTILAGE.**

A section taken from an articular surface, (from the bone of an ox) was placed in 30% solution of chromic acid, (Henke and Budge) after 2-5 min., it was washed in water, then stained in hæmatoxylin and eosin, and mounted in gum glycerin.

DRAWINGS FOR LESSON X.

DRAWINGS FOR LESSON X.

LESSON XI.

BONE.

(a) **CROSS-SECTION OF BONE.**

From a thoroughly macerated and dried bone a thin cross-section is removed from the shaft by means of a fine saw, this is ground between two hones, until it becomes very thin and transparent, care being taken to keep the stones well moistened with water, and not to use too much pressure. The section is now washed, first in distilled water and then in alcohol, this is best done by means of a fine camels-hair brush, and in a deep watch crystal. In order to determine whether your preparation is clean, mount it in a drop of alcohol and examine under the microscope, the Haversian canals and the lacunæ must not be full of sand, as they will be if the section was not well washed. If clean, remove it from the slide, place between two pieces of filter paper, and allow it to remain until perfectly dry. Bring to the table and it will be mounted for you in "hard balsam."

Examine first under low power. Observe that in a "Haversian system" the bone lamellæ are concentrically arranged about the Haversian canal. Between the lamellæ, note the bone lacunæ, these communicating with one another by means of the fine canaliculi. Look for interstitial and circumferential lamellæ.

Make a sketch of several Haversian systems as seen under low power.

(b) **LONGITUDINAL SECTION OF BONE.**

A thin longitudinal section is made from the shaft of a bone by means of a fine saw, grind and wash as cross-section, when clean allow it to dry, and it will be mounted for you.

Study first under the low power, and observe the anastomosing Haversian canals, the bone lamellæ and lacunæ are arranged parallel to them.

Make the drawing from the low power.

(c) **DECALCIFIED BONE, CROSS-SECTION.**

A small portion taken from the shaft of a fresh bone was hardened and decalcified in a solution of nitric and hydrochloric acid, (HNO_3 , 10% and HCl 1%, equal parts) thoroughly washed in flowing water, cross-sectioned, and the sections were stained in 1% sol. of acid fuchsin. They are to be mounted in gum glycerin.

Examine first under low power and observe the resemblance to the section of "hard bone;" under high power the bone corpuscles will be seen in the lacunæ.

Study also the periosteum.

(d) **LONGITUDINAL SECTION OF DECALCIFIED BONE.**

This was prepared as the cross-section and is to be mounted in gum glycerin.

Compare with longitudinal section of hard bone.

DRAWINGS FOR LESSON XI.

DRAWINGS FOR LESSON XI.

LESSON XII.

DEVELOPING BONE AND BONE MARROW.

(a) **DEVELOPING BONE.**

One of the developing bones removed from a foetal limb was hardened and decalcified in picro-suphuric acid, embedded in celloidin, sectioned and stained in hæmatoxylin and eosin. Sections are now in oil of bergamot, and need to be mounted in Canada balsam.

Section is to be studied first under the low power, and if it comes from the centre of the bone, it will show the following areas, named in order as they present themselves when the section is moved from the articular surface toward the middle of the shaft, where ossification is most advanced.

- 1). Area of articular cartilage ;
- 2). Area of flattened cartilage cells, arranged in rows ;
- 3). Area of enlarged cartilage cells, calcification may have taken place in the matrix between the rows ;
- 4). Area of ossification, osseous substance is being deposited by the "osteoblasts," on the calcified trabecullæ of the cartilage. In the spaces between the trabecullæ, (the primary marrow spaces) small arteriols and capillaries, surrounded by marrow cells, are found. The *peri-ostial bone* is being formed, and is well "marked off" from the *endochondral bone*.

Make a sketch as seen under the low power, bringing out in your drawing the several areas mentioned above.

(b) **RED MARROW.**

A small portion of red marrow taken from the femur of a young kitten is teased in normal salt solution. Study under high power. Search the preparation for nucleated red blood cells.

(c) **RED MARROW—STAINED AFTER ARNOLD'S METHOD.**

Red marrow from a kitten was placed in a test tube, containing a small quantity of normal salt solution which had been colored with methylgreen. In this solution the marrow was vigorously shaken until the cells were isolated. Place a drop of this fluid, containing some of the isolated cells, on a slide, cover and examine under high power. This preparation will enable you to differentiate the various kinds of cells normally found in red marrow.

Make a sketch of the following types, as seen under high power :

- 1). A mononuclear giant cell ;
- 2). Polynuclear giant cell, (nucleus may be lobulated), S-shaped or ring formed ;
- 3). Nucleated red corpuscle, with spherical nucleus which takes the stain very freely ;
- 4). Erythroblasts, a little larger than nucleated red blood cells containing none or very little hæmaglobin ;
- 5). Various forms of white blood cells, polynuclear, transitional, mononuclear, and perhaps a few lymphocytes ;
- 6). Myelocytes, or red marrow cells, mononuclear cells, a little larger than the white blood corpuscles.

(d) **MARROW OF GUINEA PIG, STAINED IN EHRLICH'S NEUTROPHILE MIXTURE.**

Red marrow taken from the femur of a Guinea pig was spread out on a cover-glass, fixed by exposing to a heat of 110° C. for 15 min., then stained in "Ehrlich's neutrophile mixture." Bring to the table a slide and the preparation will be mounted for you.

Examine under high power and search for the cells found in bone marrow.

A specimen will be demonstrated under the 1-12 inch oil immersion, notice the large eosinophile cells, (with red granulation); myelocytes, with the neutrophile granulation.

DRAWINGS FOR LESSON XII.

DRAWINGS FOR LESSON XII.

LESSON XIII.

VOLUNTARY OR STRIPED MUSCLE.

(a) **STRIPED MUSCLE OF FROG, FRESH.**

Tease a small shred of muscle taken from the leg of a frog in normal salt solution. Study first under low power. Observe the long cylindrical fibres, showing a transverse striation. Move the slide about and you will find broken fibres, the broken ends often united by the sarcolemma.

Cause a few drops of acetic acid (1% sol.) to flow under the cover glass, and in a few moments the muscle nuclei will be seen.

Make a sketch showing sarcolemma, also a small segment of a muscle fibre showing muscle nuclei as seen under high power.

(b) **STRIPED MUSCLE OF CAT.**

A 0.5% solution of osmic acid was injected into one of the skeletal muscles of a cat, in 30 minutes the muscle was removed, washed in flowing water, and is to be teased and mounted in gum glycerin.

Observe the structure of striped muscle fibre.

(c) **BRANCHED STRIPED MUSCLE FIBRES.**

The posterior, the free end of a frog's tongue was macerated for several hours in "M. Schultz' Mixture," a small portion is to be teased in gum glycerin.

When a voluntary muscle fibre is inserted into a mucous membrane or the epidermis, the end so inserted is often branched.

This preparation, if you have been careful in teasing it, will show you these branched fibres.

Sketch one as seen under the high power.

(d) **CROSS SECTION OF VOLUNTARY MUSCLE.**

One of the eye muscles of a dog was hardened in a saturated watery solution of bichloride of mercury, stained in borax-carmin, embedded in paraffin, and cross-sectioned. Sections are to be fixed to slide by means of the "albumen fixative," the paraffin removed and mounted in balsam.

Study first under low power. Observing the peri and endomysium. Under high power note the structure of the muscle fibres, showing the areas of Cohnheim. Observe the position of the muscle nuclei. Sketch as seen under this power.

(e) **INJECTED VOLUNTARY MUSCLE.**

Longitudinal sections of an injected voluntary muscle which had been hardened in alcohol, and embedded in paraffin, were made. Fix sections to slide by means of the "albumen fixative," remove paraffin and mount in Canada balsam.

Observe the network of injected capillaries. The fibres are not stained. Sketch as seen under low power.

(f) **DEMONSTRATION OF MOTOR ENDINGS IN VOLUNTARY MUSCLE.**

Preparation was made from one of the eye muscles of a young rabbit, the method suggested by Ehrlich, of injecting a 1% solution of methylen blue into the circulation, was used.

DRAWINGS FOR LESSON XIII.

DRAWINGS FOR LESSON XIII.

LESSON XIV.

INVOLUNTARY AND CARDIAC MUSCLE.

(a) INVOLUNTARY MUSCLE, TEASED.

The muscular coat of the small intestine of a cat was macerated for 15 minutes in a 30% solution of caustic potash, the tissue was then placed in a saturated solution of acetate of potassium (to arrest the maceration), was washed in water, and is now in gum glycerin. Observe the long, fusiform or spindle-shaped cells, the protoplasm often showing a longitudinal striation and possessing a rod-shaped nucleus.

Sketch several as seen under high power.

(b) SECTION OF INVOLUNTARY MUSCLE.

The muscular coat of the small intestine of a cat was hardened in absolute alcohol, stained in borax-carmin, embedded in paraffin, and sectioned. Fix the section to slide by means of the albumen fixative, remove the paraffin and mount in balsam.

In your preparation the fibres in the longitudinal coat will appear in cross section; note that they are of different sizes, and only a few seem nucleated, (many cross-sections are made of a cell, only one of which may pass through the nucleus).

The circular layer is cut in the direction of the long-axis of the cells. Make a sketch as seen under high power, including in your drawing a small portion of both the circular and longitudinal coats.

(c) ISOLATED HEART MUSCLE CELLS.

Small pieces of the cardiac muscle of a dog were macerated in 30% solution of KOH for 15 minutes, maceration

interrupted by placing the tissue in a saturated solution of potassium acetate, tease in gum glycerin.

Observe the short oblong cells, one end being usually branched. Cells show a cross-striation, and possess one, occasionally two, oval nuclei.

Draw several as seen under high power.

(d) **SECTION OF HEART MUSCLE.**

The ventricle of a rabbit's heart was hardened in a saturated watery solution of picric acid, stained in picrocarmin, embedded in paraffin and sectioned. Fix section to slide and mount in balsam. Note how the cells are cemented together into fibres, these into bundles.

Make drawing as seen under the high power.

DRAWINGS FOR LESSON XIV.

DRAWINGS FOR LESSON XIV.

LESSON XV.

MEDULLATED AND NONMEDULLATED NERVE FIBRES.

(a) **MEDULLATED NERVE FIBRES.**

Tease a piece from the sciatic nerve of a frog in normal salt solution, before covering arrange the fibres as straight as possible. Examine under high power, employing one of the smaller openings in the diaphragm.

Observe the axis cylinder, seen as a light band passing down through the centre of the fibre, surrounded by a thin, glistening layer, usually of a light green color, the *medullary* sheath, around this the neurolemma. Find the nodes of Ranvier, search for the nucleus of an internodal segment. Some of the fibres may show the segments of Lanterman.

(b) **MEDULLATED NERVE FIBRE STAINED IN OSMIC ACID.**

The sciatic of a frog was fixed in a 1% solution of osmic acid, tease very carefully in gum glycerin. Examine under high power.

The medullary sheath is stained deeply black by the osmic acid. The "nodes of Ranvier" are very clearly seen in this preparation.

(c) **NONMEDULLATED FIBRES.**

The splanchnic nerves of a dog were macerated in a very weak solution of chromic acid (0.01%), tease and mount in a drop of methylen blue. Examine under high power. Among the medullated fibres a few will be found that do not have a medullary sheath, showing no "nodes of Ranvier," possessing numerous nuclei, this often gives them a "beaded" appearance, these are the nonmedullated fibres.

Sketch a few nonmedullated fibres as seen under the high power.

(d) **CROSS SECTION OF A NERVE TRUNK.**

A posterior tibial nerve (human) was hardened in a saturated picric acid solution, embedded in celloidin, sectioned and stained in hæmatoxylin. Sections are now in oil of bergamot, mount in balsam. Study first under low power; note how the funiculi are held together by a loose connective tissue, the epineurium; in it groups of fat cells and the blood vessels of the nerve trunk are found. Each funiculus is surrounded by a dense connective tissue sheath the perineurium, this shows a lamellar structure. Under the high power nerve fibres in cross-section should be studied. Between the nerve fibres of a funiculus a small amount of connective tissue is seen, the endoneurium.

Draw under low power a number of funiculi and the surrounding peri and epineurium; under high power a small portion of a funiculus showing the fibres in cross-section.

(e) **DEMONSTRATION OF THE FIBRILAE OF THE AXIS CYLINDER.**

A small nerve trunk was hardened in a 1% solution of osmic saturated with picric acid, embedded in paraffin, sectioned and stained in Bœhmer's hæmatoxylin. The preparation will be shown under the one-twelfth inch oil immersion and will show the fibrilae of the axis cylinder, between which a small amount of neuroplasma is found.

DRAWINGS FOR LESSON XV.

DRAWINGS FOR LESSON XV.

LESSON XVI.

NERVE CELLS FROM SPINAL CORD, BRAIN AND PERIPHERAL GANGLION.

(a) NERVE CELLS FROM SPINAL CORD.

The gray matter was dissected from the cervical cord of an ox, macerated for several days in a very weak solution of chromic acid (1-15,000), was then stained in lithium-carmin. Tease very carefully in gum-glycerin, controlling your results under the low power. Aim to isolate several cells from the surrounding tissue. The worth of this preparation will depend largely on the care with which you have teased it.

Examine under high power and observe the large branching nerve cells; try to make out the axis cylinder process. Make a sketch of several cells as seen under this power.

(b) NERVE CELLS FROM THE POSTERIOR ROOT GANGLION.

A small piece of posterior root ganglion was fixed in a 1% solution of osmic acid, macerated in Ranvier's alcohol for several days, is now to be teased in a few drops of a 1% solution of methylenblue, in which allow it to stain five to ten minutes; wash away excess of stain with distilled water, and mount in gum-glycerin, completing the teasing in gum-glycerin if necessary.

Examine under high power, notice the spheriodal or oval ganglion cells, possessing a large spherical nucleus and nucleolus. Many of the cells may show the nucleated capsule. If you have been careful in your teasing some of the cells will show the axis cylinder process, and you may see the T-shaped junction with the nerve fibre.

Draw one cell as seen under the high power.

(c) **SECTION OF POSTERIOR ROOT GANGLION.**

The posterior root ganglion of a dog was fixed for twenty four hours in a 10% solution of nitric acid, then hardened in Müller's solution for several days, embedded in celloidin; sectioned, stained in a 1% solution of acid fuchsin. Sections are now in oil of bergamot, mount in balsam.

Study first under low power, noting the firm connective tissue capsule about the ganglion, continuous with the epineurium of the in-coming and out-going nerve trunk. From the capsule connective tissue septa pass into the interior of the ganglion.

Collection of nerve cells are seen between the group of nerve fibres. Under high power observe the structure of the ganglion cells, with their nucleated capsules.

Make a sketch of the ganglion as seen under the low power.

(d) **DEMONSTRATION OF THE CORTEX OF CEREBRUM, CEREBELLUM AND SPINAL CORD, STAINED AFTER GOLGI'S METHOD.**

Sections were made from an embryo pig, and in them only a few of the nerve cells are stained.

Observe the pyramidal cells of the cortex, Pürkinje's cells in the cerebellum, the motor (typus I of Golgi) and the sensory (typus II of Golgi) in the spinal cord.

DRAWINGS FOR LESSON XVI.

DRAWINGS FOR LESSON XVI.

LESSON XVII.

SPINAL CORD AND BRAIN.

(a) SECTION OF SPINAL CORD.

A human cord was hardened in Müller's solution, embedded in celloidin and double stained in nigrosin and eosin. Sections are now in the oil of bergamot, mount in balsam. It will not be possible for the student to obtain more than a general idea of the structure of the brain and spinal cord in this lesson; many sections which need be especially stained are required to bring out the finer anatomy of the central nervous system.

Examine under low power, and note the arrangement of the gray and white matter. The former appears in cross-section, in form of two crescents, the convex borders of which are united by a commissure composed of white (anteriorly) and gray (posteriorly) matter.

The anterior horns of the crescents are broader and shorter and do not come so near to the surface as the posterior.

The white matter surrounds the gray, and is composed of medullated nerve fibres, seen in cross-section, between these fibres a small amount of *neuroglia tissue* is observed.

In the gray matter a very fine network of fibres is seen, composed of medullated fibres, of naked axis cylinders, branches of nerve cells, and of neuroglial tissue. The nerve cells are found in groups in the anterior and posterior horn.

Sketch a small portion of the gray and white matter as seen under high power.

(b) SECTION OF CORTEX.

A portion of the cortex taken from a brain (human)

was hardened in Müller's solution, embedded in celloidin sectioned, stained in neutral carmin (Fritsch's method). Sections are now in the oil of bergamot; mount in balsam. Under high power the following layers will be "made out" in the cortex :

1). The outer or molecular layer, composed largely of neuroglial tissue, in it a few small nerve cells and a thin stratum of fine medullated fibres, running parallel to the surface, and found just under the pia mater, are seen.

2 and 3). Layer of small and large pyramidal cells, in the former the cells are small and close together, in the latter they are larger and farther apart, there is, however, no distinct boundary line between the two layers.

4). A layer of small cells, some of which are pyramidal, others spindle-shaped, others multipolar.

Sketch a small segment of the cortex as seen under high power.

(c) SECTION OF CEREBELLUM (HUMAN.)

Hardened in Müller's fluid, embedded in celloidin, sectioned and stained in nigrosin and eosin. Sections are now in oil of bergamot, mount in balsam. Study first under low power, observing the folded appearance of the cortex.

Under high power the gray matter shows the following layers in cross-section.

1). An outer molecular layer composed largely of neuroglial tissue, containing a few small ganglion cells.

2). Between the above stratum and the third a single layer of large ganglion cells, *Purkinje's cells* are found; from the base of these cells an axis cylinder process is given off; from the opposite pole one or two protoplasmic processes; these extending into the molecular layer, there dividing and redividing, until the processes have the appearance of a deer's antlers.

3). The granular layer, composed largely of round and spindle-shaped cells, possessing comparatively large

nuclei, so that in the section very little but the nuclei will be seen. The axis cylinder process of Pürkinje's cells pass through this layer, become medullated and are lost in the white substances found making up the central portion of the fold.

Sketch the cortex of the cerebellum as seen under high power.

DRAWINGS FOR LESSON XVII.

DRAWINGS FOR LESSON XVII.

DRAWINGS FOR LESSON XVII.

LESSON XVIII.

ARTERIES, VEINS AND ADENOID TISSUE.

(a) CROSS SECTION OF ARTERY AND VEIN.

The posterior tibial artery and vein (human) were hardened in picric acid, embedded in celloidin, sectioned, stained in Boehmer's hæmatoxylin. Sections are now in the oil of bergamot, mount in balsam.

Observe first under low power; in the wall of the artery three coats are seen.

1). An inner coat, the "*tunica intima*," consisting of the following structures: A single layer of endothelial cells lining the lumen of the artery; a thin stratum of sub-endothelial connective tissue; a stratum of elastic tissue, the so called elastic intima or the fenestrated layer of Henle.

2). Middle coat, the "*tunica media*," composed largely of circularly disposed bundles of nonstriped muscle tissue, between these thin films of elastic tissue, seen in cross-section as wavy lines, are found.

3). Outer coat or "*tunica adventitia*," consists of bundles of white fibrous tissue, felted into a dense layer. Between these bundles a few elastic fibers are seen. This coat is continuous with the surrounding connective tissue.

The wall of the vein is not so thick, three coats are seen, resembling in structure the ones observed in the artery.

In the intima of the vein the elastic layer is not so prominent; in the tunica media there is relatively less muscular tissue, the bundles of which are separated by white fibrous tissue. The adventitia is thicker than the corresponding coat in the artery. This preparation also

shows in section a number of small arteries and veins in the fibrous tissue surrounding the larger vessels.

Make a sketch of a segment of the wall of the artery, also of a segment of the wall of the vein, and of a small artery as seen under low power.

(b) **CROSS-SECTION OF THE AORTA.**

The aorta of a dog was hardened in picric acid, embedded in celloidin, and stained in lithion-picro-carmin. Sections are now in the oil of bergamot, mount in balsam. Study first under low power. The media is very well developed, between the muscular elements, a large amount of elastic tissue is found. Sketch as seen under low power.

(c) **CAPILLARIES.**

Study preparation of teased spinal cord for isolated capillaries. Sketch several as seen under high power.

(d) **ADENOID TISSUE.**

The vermiform appendix of a dog was hardened in alcohol, stained in borax carmin, embedded in paraffin, sectioned. Fix sections to slide and mount in balsam. Examine under high power. In a section of adenoid tissue very little is seen of the reticular network; the interstices of the retiform tissue are so full of cells that it is obscured.

DRAWINGS FOR LESSON XVIII.

DRAWINGS FOR LESSON XVIII.

LESSON XIX.

LYMPHATIC GLAND, SPLEEN AND THYMUS.

(a) COMPOUND LYMPH GLAND.

A lymphatic gland was hardened in picric acid stained in lithion-picro-carmin, embedded in paraffin, sectioned. Fix sections to slide and mount in balsam. Examine first under low power. Observe the capsule, from which trabecullæ pass into the gland, dividing its outer or cortical portion into comparatively large compartments, in which the *cortical nodules* of adenoid tissue are found. On entering the medulla the trabecullæ divide and anastomose, the network so formed has small meshes, in which are found the *medullary cylinders* of adenoid tissue. The adenoid tissue is composed of a reticular frame work and cells.

“The cortical follicles and the medullary cylinders do not completely fill out the compartments made for them by the capsule and trabecullæ respectively, but a narrow peripheral zone of each compartment is left free, this is a lymph sinus.”—(Klein.) The lymph sinuses form an anastomosing system.

Sketch a portion of a gland as seen under low power.

(b) RETICULUM OF ADENOID TISSUE.

A lymph gland was hardened in alcohol, cut on the freezing microtome. Sections were stained in Boehmer's hæmatoxylin. They were then transferred to a test-tube half full of distilled water, in which they were carefully shaken for ten or fifteen minutes. In this way many of the lymph cells are “shaken out” of the reticulum. Mount the preparation in gum glycerin. Examine under

high power, using one of the smaller openings in the diaphragm.

A reticulum of very fine fibrils will be seen, fixed connective tissue cells are often found on the network. Sketch as seen under this power.

(c) **SECTION OF SPLEEN.**

Small pieces from the spleen of a dog were hardened in bichloride of mercury, stained in borax-carmin, embedded in paraffin, and sectioned. Fix sections to the slide; remove paraffin and mount in balsam.

Observe the capsule composed of connective tissue and non-striped muscle cells. From the capsule, trabeculae pass into the gland, branching and forming an anastomosing network; they are in structure like the capsule. Within the capsule two kinds of tissue are found, the Malpighian corpuscle and the spleen pulp.

The former are composed of adenoid tissue, this is usually found surrounding an artery. In the pulp a frame-work of fine fibrils and cells is found, in the meshes of which red and white blood cells are seen.

Make a sketch of a portion as seen under high power.

(d) **DEMONSTRATION OF SECTION FROM THYMUS GLAND.**

DRAWINGS FOR LESSON XIX.

DRAWINGS FOR LESSON XIX.

LESSON XX.

SKIN AND ITS APPENDAGES.

(a) **MACERATED EPIDERMIS.**

A small piece of skin was macerated for several days in a 0.25 % solution of acetic acid. The epidermis was then carefully lifted from the dermis and stained in Bøehmer's hæmatoxylin. The fragments of epidermis are now in oil of bergamot, mount in balsam. Care being taken to so place the tissue on the slide that the under surface of the epidermis is presented to the observer. On studying under low power you will see the depressions in the under surface of the epidermis, into which the papillæ of the true skin fit.

(b) **MACERATED PREPARATION OF THE DERMIS.**

The skin was macerated as above; on the freezing microtome, the dermis was cut into quite thick transverse sections, these were stained in Bøehmer's hæmatoxylin, are now in the oil of bergamot; mount in balsam.

Section is given to demonstrate the papillæ of the true skin, in many the capillary net-work can be made out.

Make a drawing of a number of papillæ as seen under the low power.

(c) **CROSS-SECTION OF SKIN, (HUMAN.)**

A portion of the skin removed from the plantar surface of the foot was hardened for twenty-four hours in 10% nitric acid, then in Müller's fluid, embedded in celloidin and sectioned, double stained in hæmatoxylin and eosin. Sections are now in the oil of bergamot; mount in balsam.

Section is to be studied first under low power, and the general arrangement of the tissues observed.

In the epidermis the following layers are described, named in order from within, outwards.

1). Stratum Malpighi, (rete mucosa) composed of stratified pavement epithelium.

2). Stratum granulosum, a narrow layer, the cells of which contain keratohyalin granules (Waldeyer) in their protoplasm.

3). Stratum lucidum, composed of horny cells the outlines of which are very indistinct.

4). Stratum corneum, the thick outer layer.

Observe also the twisted portion of the sudoriferous glands, passing through the epidermis.

In the cutis vera an outer denser portion, the papillary layer is observed, composed of closely interwoven bundles of white fibrous tissue, between which a small amount of elastic tissue is found.

This layer is beset with papillæ.

In the deeper portion of the corium, the reticular layer, the bundles of fibrous tissue are loosely woven.

In the large open meshes groups of fat cells and the secreting portion of the sweat glands are found.

Some of the preparations may also show a Vater-Pacinian corpuscle in section.

Make a sketch of the several layers of the epidermis and dermis as seen under low power.

(d) **CROSS-SECTION. OF THE SCALP.**

A portion of the scalp was hardened in Müller's fluid, embedded in celloidin, sectioned and stained in hæmatoxylin and eosin. Sections are in oil of bergamot; mount in balsam.

In this preparation especial attention is to be given to the study of the hair and its follicle. An attempt was made to cut them longitudinally.

Examine first under low power, and observe the sev-

eral layers of the follicle, the sebaceous gland in connection with it, and the arrectores pili.

Sketch as seen under this power.

(e) **TANGENTIAL SECTION OF SCALP.**

Tissue was hardened in Müller's fluid, sections were double-stained in hæmatoxylin and eosin; are now in oil of bergamot; mount in balsam.

In this preparation the hair follicles are seen in cross-section. Study under high power. Each follicle shows the following layers, named in order from without, in:

In the dermal coat:

- 1). A longitudinal fibrous layer.
- 2). A circular fibrous layer.
- 3). Glassy layer.

In the epidermal coat:

- 1). Outer root sheath (rete mucosa).
- 2). Henle's layer.
- 3). Huxley's layer.
- 4). Cuticle of the shaft.

Sketch one follicle as seen under high power.

(f) **DEMONSTRATION OF MEISSNER'S TACTILE CORPUSCLE,**

In a preparation stained with chloride of gold.

(g). **DEMONSTRATION OF LONGITUDINAL SECTION THROUGH
THE THIRD PHALANX OF A TOE,**

Showing nail in position.

DRAWINGS FOR LESSON XX.

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DRAWINGS FOR LESSON XX.

CHICAGO MEDICAL SCHOOL

DRAWINGS FOR LESSON XX.

LESSON XXI.

TONGUE, TASTE-BUDS, SALIVARY GLANDS.

(a) CROSS-SECTION OF TONGUE.

A cat's tongue was hardened in picric acid, embedded in celloidin, sectioned and stained in lithion-picro-carmin. Sections are in the oil of bergamot, mount in balsam. Preparation is given to show the structure of the papillæ on the dorsum of the tongue, and the arrangement of the muscle fibres in the tongue. The lingual artery and nerve will be seen in cross-section. Sketch a number of the papillæ as seen under low power.

(b) STRUCTURE OF TASTE-BUDS.

The papillæ foliata of a rabbit was hardened in Fleming's solution, stained in alum-carmin and embedded in paraffin. Fix sections to slide, and mount in balsam. The sections were cut at right angles to the transverse ridges found in the papilla foliata. In the epithelium lining the furrows between the ridges, the taste buds are embedded. In the muscular and connective tissue below the papilla foliata, small serous glands will be seen in section.

Sketch two ridges, with the intervening furrow, (in the walls of which the taste-buds are found,) as seen under high power.

(c) SUBMAXILLARY GLAND.

Submaxillary gland of a dog was hardened in absolute alcohol, stained in Haidenhain's hæmatoxylin, embedded in paraffin, and sectioned. Fix sections to slide and mount in balsam.

Under low power observe how the acini are grouped into lobules, these are loosely bound together by connec-

tive tissue. Inter and intra-lobular ducts will be seen in section showing the striated epithelium. Under high power the structure of the alveoli is to be carefully studied. They are surrounded by a membrana propria, and nearly filled with clear mucous cells, the nucleus of which is found in their peripheral portion. Many of the acini will show a crescent of more deeply stained cells, the demilunes of Haidenhain or crescents of Guianuzzi, between the mucous cells and the membrana propria.

Sketch an intra-lobular duct with a number of the surrounding alveoli as seen under high power.

(d) **PAROTID GLAND.**

Pieces from parotid gland of a dog were hardened in absolute alcohol, stained in Haidenhain's hæmatoxylin, embedded in paraffin. Fix the section to slide and mount in balsam.

Under low power the same general structure as observed in studying sub-maxillary gland will be seen. Under high power the acini are found to be a little smaller, and are lined by a cubical or polyhedral cells, showing a granular protoplasm.

Sketch a few alveoli as seen under high power.

(e) **OESOPHAGUS.**

The œsophagus of a dog was hardened in a 10% nitric acid for twenty-four hours, then in Müller's fluid for several days. Embedded in celloidin, cross-sectioned and stained in Bœhmer's hæmatoxylin and eosin, are now in oil of bergamot. Mount in balsam. Study first under low power and the following coats will be observed. The œsophagus is lined by a stratified pavement epithelium, this resting on a papillated mucosa, which is limited externally by a muscularis mucosa. Then follows the sub-mucosa, a fibrous tissue coat, containing the larger vessels. Your section may show mucous glands in this stratum.

Next a muscular coat, composed of inner circular and

outer longitudinal bundles. If section is from the upper third the muscular tissue is largely of the striped variety, below this non-striped.

Sketch a segment of the wall as seen under low power.

DRAWINGS FOR LESSON XXI.

DRAWINGS FOR LESSON XXI.

DRAWINGS FOR LESSON XXI.

LESSON XXII.

INTESTINAL CANAL.

[a) **CROSS-SECTION OF THE WALL OF CARDIAC END OF STOMACH.**

A segment from the wall of the cardiac end of the stomach of a dog was hardened in absolute alcohol, stained in borax carmin, embedded in paraffin, and sectioned. Fix sections to slide and mount in balsam.

The study of the intestinal canal will be much simplified, if the student bears in mind, that through its whole extent (excepting the œsophagus, and the lower part of the rectum, where no outer serous coat is found) it is composed of four coats. The outer three of which, namely: the serous or peritoneal, the muscular, (composed of an outer longitudinal and an inner circular layer), and the sub-mucosa, or the connective tissue coat, are very similar in structure. The structural differences observed in the several anatomical divisions of it, are found in the mucosa, and especially in the glands of this coat.

Study the sections first under low power, the finer details being made out under the high. The simple or compound tubular glands found in the mucosa of the cardiac end of the stomach, show a short duct and a comparatively long secreting tubule. In the latter two kinds of cells are found, the *central or chief cells*, short columnar or polyhedral in shape, lining the lumen, and the *parietal or oxyntic cells* of Langley, of oval shape.

Make a sketch, reproducing the several coats, as seen under lower power.

(b) **MUCOUS MEMBRANE OF CARDIAC END.**

The mucous membrane of the cardiac end of the stomach was hardened in absolute alcohol, stained in

Haidenhain's hæmatoxylin, embedded in paraffin and cross-sectioned. Fix to slide and mount in balsam. Study under high power. In the preparation the structure of the cardiac glands can be more easily made out, the chief cells having taken a steel-gray color, while the oval cells are stained black.

Sketch one gland as seen under high power.

(c) **PYLORIC END OF STOMACH.**

The tissue was taken from the pyloric end of a dog's stomach, hardened in alcohol, stained in borax carmin, embedded in paraffin. Fix sections to slide and mount in balsam. Observe that in the pyloric glands the duct is longer, the secreting tubule correspondingly shorter, and lined by only one kind of cells, these resembling the chief cells in the cardiac glands. Your section may show small masses of adenoid tissue, found in the deeper portion of the mucosa.

Sketch two pyloric glands as seen under low power.

(d) **SMALL INTESTINE.**

The small intestine of a dog was hardened in absolute alcohol, stained in borax carmin, embedded in paraffin, mount in balsam.

Two specimens are given, one of which contains a solitary gland in section. Note the villi, conical projections of the mucosa, covered by a single layer of columnar cells. Observe the chyle vessel in the center of each villus. In the mucosa are found many tubular glands, the crypts of Lieberkühn, coming to the surface between the villi. In the section showing the solitary gland the lymphoid tissue is found in submucosa and mucosa.

Sketch a small segment of the section not containing the solitary gland as seen under low power, reproducing in your drawing the several coats.

(e) **INJECTED SMALL INTESTINE.**

The mesenteric artery of a cat was injected with gela-

tin-carmin, hardened in alcohol, embedded in paraffin and sectioned, mount in balsam. The section is not stained. Observe under low power, and note the arrangement of the injected vessels in the muscular coat, in the submucosa, about the crypts of Lieberkühn, and in the villi.

(f) **DEMONSTRATIONS OF AUERBACH'S AND MEISSNER'S
PLEXUS IN THE RECTUM OF A FROG.**

Methylenblue, (Ehrlich.)

DRAWINGS FOR LESSON XXII.

DRAWINGS FOR LESSON XXII.

DRAWINGS FOR LESSON XXII.

LESSON XXIII.

LARGE INTESTINE, LIVER AND PANCREAS.

(a) **LARGE INTESTINE.**

Large intestine of dog was hardened in alcohol, stained in borax-carmin, embedded in paraffin, and cross-sectioned mount in balsam. Notice that in the large intestine no villi are found. Simple tubular glands, the crypts of Lieberkühn, (lined by a single layer of short columnar cells, the great majority appearing as goblet cells,) placed vertically in the mucosa are observed.

Sketch the section as seen under high power.

(b) **LIVER CELLS.**

Small pieces from the liver of a cat, were macerated for twenty-four hours in Ranvier's alcohol, for two hours in 0.5% osmic acid. Tease in gum-glycerin. Study under high power. The liver cells are polyhedral in form, possessing a distinct intra-cellular network. Fat globules, (stained black,) may be seen in the cells. As a rule a single spherical nucleus is found.

Sketch several as seen under high power.

(c) **INJECTED LIVER.**

The liver of a pig was injected through the portal vein with Berlin-blue, hardened in alcohol, stained in borax-carmin, embedded in paraffin and sectioned; mount in balsam. Study under high power. Note that the gland is composed of lobules, the injected inter-lobular branches of the portal vein, are seen between them. From these inter-lobular vessels capillaries pass into the lobule, uniting in an intra-lobular vessel, these empty into sublobular veins. The liver cells appear arranged in columns between the capillaries.

Sketch two lobules showing the blood supply as seen under low power.

(d) **SECTION OF LIVER.**

Pieces from the liver of a cat were hardened in Müller's fluid, embedded in celloidin, stained in Boëhmer's hæmatoxylin. Sections are in oil of bergamot, mount in balsam. Study first under the low power and observe the arrangement of the liver cells in the hepatic lobules. Search for a bile duct and note that it is lined by columnar epithelium.

(e) **LIVER WITH BILE CAPILLARIES STAINED. (OPPEL'S METHOD.)**

Small pieces of liver were hardened in a solution of bichromate of potassium and osmic acid (Ramon J. Cajal,) for three days, were then transferred to 0.75% solution of nitrate of silver, in which they remained for several days. Sections are in turpentine, mount in hard balsam. The bile capillaries are stained black. Two sections are given, one from the liver of an embryo rat, in this the compound tubular character of the gland is easily made out; the second from the liver of a young kitten, showing an apparent network of bile capillaries between the liver cells.

(f) **PANCREAS.**

The pancreas of a dog was hardened in bichloride of mercury, stained in borax-carmin, embedded in paraffin. Fix sections to slide and mount in balsam. Study under high power. The structure of the pancreas is in many respects similar to that of a serous gland. Note the inner granular zone in the cells lining the alveoli, the nucleus is found in the outer zone.

Sketch several alveoli as seen under high power.

DRAWINGS FOR LESSON XXIII.

DRAWINGS FOR LESSON XXIII.

LESSON XXIV.

ORGANS OF RESPIRATION.

(a) **TRACHEA.**

The trachea of a dog was hardened for twenty-four hours in a 10% nitric acid solution, then in Müller's fluid for eight days; embedded in celloidin and cut longitudinally. Sections were stained in hæmatoxylin and eosin, are now in oil of bergamot, mount in balsam. The trachea is lined by stratified, columnar, ciliated epithelium, resting on a basement membrane. The mucosa is composed of loose areolar tissue, limited externally by an elastic layer. In the fibrous layer several of the cartilagenous hoops are seen in cross-section. Sections of small mucous glands may be observed in this layer.

Sketch as seen under high power.

(b) **LUNG.**

The lung of a cat was hardened in picric acid, embedded in celloidin, stained in Bøehmer's hæmatoxylin and eosin. Sections are now in the oil of bergamot, mount in balsam. Study first under low power and notice the bronchi, the blood vessels and the alveoli. The sections of the large bronchi, show a lining of stratified, columnar, ciliated epithelium, resting on a fibrous tissue mucosa in which small mucous glands may be observed; next a band of non-striped muscle tissue, outside of which cartilagenous plates may be seen in cross section. The smaller bronchi are lined by a *single* layer of ciliated columnar cells, and the cartilagenous plates are wanting. Try and make out the capillaries between the alveoli, a few blood cells may be seen in them.

Make a sketch of a segment from the wall of a large bronchus, and of a small bronchial with a few of the surrounding alveoli, as seen under high power.

a) **INJECTED LUNG.**

The lung of a cat was injected through a pulmonary artery, hardened in alcohol, embedded in paraffin. Fix the sections to the slide and mount in balsam. The sections are not stained. Under high power observe the injected capillary network about the alveoli.

(d) **THYROID GLAND.**

The thyroid gland was hardened in absolute alcohol, embedded in celloidin, and stained in lithion-picro-carmin. Sections are in oil of bergamot, mount in balsam.

The gland is surrounded by a fibrous tissue capsule. Oval or round alveoli, lined by a single layer of cubical cells, are seen in section.

Sketch a number of alveoli as seen under high power.

DRAWINGS FOR LESSON XXIV.

DRAWINGS FOR LESSON XXIV

LESSON XXV.

KIDNEY, ADRENAL GLAND, BLADDER.

(a) ISOLATED TUBULES OF KIDNEY.

The kidney of a small mammal was macerated for twenty-four hours in 30% solution of hydrochloric acid, washed for an hour in flowing water.

Tease very carefully in gum-glycerin.

Different portions of the uriniferous tubules will be seen in the field.

Sketch a Malpighian corpuscle, a convoluted tubule, a loop of Henle and a collecting tubule as seen under low power.

(b) LONGITUDINAL AND TRANSVERSE SECTION OF KIDNEY.

The kidney of a young rat (or other small mammal), was hardened in alcohol, stained in borax-carmin, embedded in paraffin. Fix sections to slide and mount in balsam. Two sections are given, the one was cut longitudinally the other transversely. Study under low power and observe the arrangement of the tubules in the cortical and medullary portion, as seen both in the longitudinal and transverse section. In the preparation before you a single Malpighian pyramid is found.

In the medullary portion the tubules have a more or less straight direction, radiating from the apex toward the base of the Malpighian pyramid. In the cortex, bundles of straight collecting tubules arranged in the form of pyramids, (the pyramids of Ferrein or medullary rays) the bases of which rest on the base of the Malpighian pyramid, are observed. Between the medullary rays, the labyrinth of the kidney, composed of the Malpighian corpuscles,

proximal and distal convoluted, spiral and zigzag portions of the uriniferous tubules, is found.

Under high power the form and structure of the epithelium lining the different segments of the uriniferous tubules is to be studied.

Sketch a portion of the cortex as seen under low power.

(c) **HORIZONTAL SECTION OF CORTEX.**

The kidney of a dog was hardened in Müller's fluid, stained in alum-carmin, embedded in paraffin and cut at right angles to the medullary rays. Fix to slide and mount in balsam. Under low power the medullary rays will be recognized, as round or oval masses, composed of quite small regular tubules seen in cross-section. These are surrounded by the labyrinth.

Sketch one medullary ray and a number of the convoluted tubules about it as seen under low power.

(d) **INJECTED KIDNEY.**

The kidney of a dog was injected with Berlin blue through the renal artery, hardened in alcohol, stained in borax carmin, embedded in paraffin. Fix sections to slide and mount in balsam. Study under low power, observing the injected interlobular arteries. The glomeruli with their afferent and efferent vessels, the capillary network about the convoluted tubules, and the straight capillaries, of the medulla.

(e) **ADRENAL BODY.**

The adrenal body of a rabbit was hardened in a saturated solution of bichloride of mercury, stained in borax-carmin, embedded in paraffin and cross-sectioned. Fix the sections to slide, and mount in balsam. Study first under low power, observing the arrangement of the cells in the cortex and medulla. Under high power the structure of the cells is to be made out.

Sketch a portion of the medulla and cortex as seen under low power.

(f) **BLADDER.**

The bladder of a dog was partially distended and hardened for twenty-four hours in a 10% nitric acid solution, then in Müller's fluid for several days, embedded in celloidin, cross-sectioned, and stained in hæmatoxylin and eosin. Sections are in oil of bergamot; mount in balsam. The epithelium lining the bladder is transitional, and is in structure like that lining the ureter and pelvis of the kidney. The epithelium rests on a fibrous tissue mucosa. The muscular tissue of the bladder is non striped.

Make a sketch of a portion of the preparation showing the several coats.

DRAWINGS FOR LESSON XXV.

DRAWINGS FOR LESSON XXV.

DRAWINGS FOR LESSON XXV.

LESSON XXVI.

MALE AND FEMALE GENERATIVE ORGANS.

(a) CROSS-SECTION OF TESTIS.

The testis of a rat was hardened in a saturated watery solution of picric acid, embedded in celloidin, cross-sectioned and stained in lithium-carmin. Sections are now in oil of bergamot; mount in balsam. The epididymis is included in this preparation. Study first under low power, and observe the fibrous tissue capsule, the tunica albuginea surrounding the gland, from this fine septa pass into the parenchyma, supporting the seminiferous tubules; some of these are cut transversely, others obliquely, and some longitudinally. The seminiferous tubules are lined by several layers of epithelial cells, the tubules of the epididymis by a layer of columnar ciliated cells.

This preparation is given to show the general structure of the gland.

Sketch a portion of the testis as seen under low power.

(b) SECTION OF TESTIS TO SHOW SPERMATOGENESIS.

Small pieces from the testis of a guinea-pig were hardened in Fleming's solution, embedded in paraffin. The sections were fixed to a coverglass, double stained in safrinin and licht grün. The coverglasses to which the preparation are fixed are now in xylol; mount in balsam. Study under high power, and on moving the section about you will observe that the variously cut seminiferous tubules, show different stages of development. In a resting tubule, within the tunica propria are seen several layers of cells; in the outermost of which are found the *spermatogones* (parent cells), and the lower portion of

the supporting cells of Sertoli. Then comes a layer of quite large cells the *spermatocytes* (mother cells), descendants of the spermatogones. Lining the lumen of the tubules are found several layers of small cells, the *spermatoblasts* (daughter cells). The mother cells dividing by indirect cell division give rise to the small inner cells, from them the spermatozoa are developed. Search for a tubule showing the fusion of the spermatoblasts with the supporting cells of Sertoli. Other tubules will show the successive steps in the development of the spermatozoa in these cells, until they are found free in the lumen of the tubule.

Bring out in several drawings, made of tubules showing different stages of development, the several steps of spermatogenesis as seen in your preparation.

(c) **CROSS-SECTION OF OVARY.**

The ovary of a bitch was hardened in a 10% HNO_3 solution for twenty-four hours, in Müller's fluid for several days, embedded in celloidin, stained in hæmatoxylin and eosin. Sections are now in oil of bergamot; mount in balsam. Study first under low power. The ovary is covered by a single layer of *germinal epithelium*. A medullary portion (in which large vessels are found) surrounded by a cortical portion containing the Graafian follicles seen in different stages of development, is observed. The framework or stroma of the ovary is fibrous and non-stripped muscle tissue. A fully developed Graafian follicle shows the following structure: The surrounding stroma is denser; the follicle is lined by the *membrana granulosa*, composed of several layers of small cells. In one portion of the follicles the *membrana granulosa* is thickened, the *discus proligerous*, in this discus the ovum is embedded. The cavity of the follicle is filled by the liquor folliculi. Other follicles in different stages of development will also be observed; in some the ovum is surrounded by only a single layer of cells; in others by

several layers ; while in still others the cavity of the follicle may just be forming.

Make a sketch of a number of follicles showing different stages of development as seen under the high power.

(d) **UTERUS.**

The uterus of a pig was hardened in alcohol, stained in carmin, embedded in paraffin, and cross-sections of one of the horns were made. Fix sections to slide, and mount in balsam. Study under high power. The cavity of the uterus is lined by a single layer of ciliated columnar epithelial cells. The mucosa is quite thick, shows many small vessels in section ; in it the utricular glands are found ; the muscular tissue is non striped ; observe the arrangement of the bundles.

Sketch a portion of the wall showing the several coats as seen under low power.

DRAWINGS FOR LESSON XXVI.

DRAWINGS FOR LESSON XXVI.

DRAWINGS FOR LESSON XXVI.

LESSON XXVII.

THE EYE.

a) SECTION THROUGH ANTERIOR HALF OF EYE.

The eye (human) was hardened in 10% solution of HNO_3 for 48 hours, in Müller's fluid for one week, the anterior half was embedded in celloidin, sectioned and stained in hæmatoxylin and eosin. Sections are in oil of bergamot mount in balsam. This section shows the anterior portion of the three coats of the eye in their normal relative position, and also the lens and its suspensory ligament. Study first under low power.

The *cornea* is composed of five layers, named in order from before backwards: (1) Stratified pavement epithelium; (2) Bowman's layer or the anterior homogeneous lamella; (3) Substantia propria, the thickest of the several coats, composed of bundles of white, fibrous tissue arranged in layers, between which the corneal corpuscles are found; (4) The posterior elastic or Descement's membrane, (5) The endothelium of the anterior chamber.

In the sclera the bundles of white fibrous tissue are densely woven, and are continuous with the fibrous tissue bundles of the substantia propria of the cornea, but are not so regularly arranged. Observe the canal of Schlemm as seen in cross-section in the sclero-corneal junction. Of the middle layer the iris, ciliary body and anterior portion of the choroid are included in this section. The iris is covered anteriorly by a layer of cells continuous with the ones found on the posterior surface of the cornea. The Stroma is a loose fibrous tissue in which pigmented and unpigmented branched cells and many vessels are found. The iris is

covered posteriorly by a double layer of deeply pigmented cells, the *pars retinae iridis*. The fibres of the sphincter of the iris are seen in cross-section near its free edge and posterior surface. Observe the *ligamentum pectinatum* (composed of trabecullæ of fibrous tissue) uniting the ciliary body to the outer coat at the sclero corneal junction. The spaces between the trabecullæ of this ligament communicate with the anterior chamber and are known as *Fontana's spaces*. The *ciliary body* is a very much thickened portion of the middle coat, is continuous, anteriorly with the iris and posteriorly with the choroid. It is composed of meridionally placed folds, the *ciliary processes* and the *ciliary muscle*. The meridional fibres of this muscle have their origin from the wall of the canal of Schlemm and the adjacent fibrous tissue, extending backward to the anterior portion of the choroid, (the tensor choroideæ,) and into the ciliary body. The equatorial or the circular fibres of Müller, are observed in cross-section near the base of the iris. The ciliary body and processes are covered by a double layer of pigmented cells, the *pars ciliaris retinae*. Only a small portion of the choroid is seen in this section. Note how vascular it is. In the stroma many branched and pigmented connective tissue cells are seen.

Notice how the coats of the retina are quite abruptly reduced to a double layer of cells at the ora serata, continuing over the ciliary body and posterior surface of the iris as *pars ciliaris retinae* and *pars iridis retinae*. The lens is enclosed in a homogeneous capsule. The *substantia propria* of the lens consists of long, nucleated cells (the lens fibres) arranged in layers. The section may show the single layer of short cubical cells, found on the anterior surface of the lens just under the capsule. Observe the suspensory ligament of the lens, composed of homogeneous fibres, these seemingly arising from the apexes of the ciliary processes and passing from these to the equator of the lens, some uniting with the capsule of the lens on its

anterior, others on its posterior surface. Make a drawing of this preparation as seen under the low power.

(b) **LENS FIBRES.**

A lens was macerated for several days in 0.5% solution of HCl. Tease and mount in gum glycerin, and examine under the high power.

Sketch several fibres as seen under this power.

(c) **RETINA.**

A hardened retina was stained in hæmatoxylin, embedded in paraffin, and cross-sectioned. Fix sections to slide and mount in balsam. Study under the high power. The following layers are made out, named in order from before backwards. (1) Internal limiting membrane; (2) layer of nerve fibres; (3) layer of ganglion cells; (4) inner granular or molecular layer; (5) the inner nuclear layer; (6) Outer granular or molecular layer; (7) Outer nuclear layer; (8) Outer limiting membrane; (9) layer of rods and cones; (10) layer of pigment cells, this layer often remains attached to the choroid and may therefore not show in your section).

Sketch the retina as seen under high power.

(d) **DEMONSTRATION.**

A section through the *fovea centralis*, from human eye.

(e) **DEMONSTRATION.**

Section through the coats of an eye at the point of entrance of the optic nerve.

DRAWINGS FOR LESSON XXVII.

DRAWINGS FOR LESSON XXVII.

DRAWINGS FOR LESSON XXVII.

LESSON XXVIII.

COCHLEA AND OLFACTORY MUCOUS MEMBRANE.

(a) COCHLEA OF GUINEA PIG.

The cochlea of a Guinea pig was hardened in Fleming's solution, decalcified in 1% chromic acid embedded in celloidin. Sections were cut in a direction parallel to the long axis; were stained in hæmatoxylin and acid fuchsine and are now in oil of bergamot, mount in balsam. The cochlear canals were opened on one side so that the hardening fluid might penetrate more easily. Study first under low power. Observe the bony axis, the modeolus, about which the cochlear canal is spirally wound. Sections of it are seen on each side of the modeolus. The cochlear canal is divided into two portions by the *lamina spiralis* (a bony crest attached to the modeolar wall of the canal) and the *basilar membrane*, extending from the lamina spiralis to the *ligamentum spiralis*. The upper portion of the canal is the *scala vestibuli*, the lower the *scala tympani*; they are lined by endothelial cells. A triangular canal, the cochlear duct or scala media is cut off from the scala vestibuli by Reissner's membrane. In the cochlear duct resting on the basilar membrane is found the *organ of Corti*, in which the following parts are to be distinguished: The pillars of Corti, arranged in the form of an arch; the inner and the three or four outer hair cells; Deiter's cells supporting the outer hair cells; peripheral to the organ of Corti; Hensen's cells, in these cells fat granules are found in the guinea pig. Note the *membrana tectoria* resting on the organ of Corti. Observe the spiral ganglion in the lamina spiralis, from this nerve fibres pass toward the organ of Corti and make con-

nection with the hair cells. They may be seen passing through the tunnel of Corti.

Sketch the preparation as seen under low power, and a cochlear duct with the organ of Corti as seen under the high.

(b) **OLFACTORY MEMBRANE (TEASED).**

The olfactory membrane of a frog was fixed and macerated in a 0.5% solution of osmic acid. Place a small piece of the macerated tissue on a slide and cover with a few drops of methylenblue, allow it to stain for several minutes, wash away excess of stain with distilled water, add a drop of gum-glycerin, tease and mount. Study under high power and search for *olfactory* and *sustentacular cells*.

Sketch a number of the olfactory cells as seen under this power.

(c) **OLFACTORY MEMBRANE.**

The mucous membrane was removed from the septum of a rabbit's nose, hardened in Fleming's solution, stained in hæmatoxylin and embedded in paraffin; fix sections to the slide and mount in balsam.

This preparation shows olfactory and respiratory mucous membrane. The epithelium covering the latter is stratified, ciliated, columnar, in the regio olfactoria the two kinds of cells studied in the teased preparation will be observed. In the mucosa, which is composed of loose fibrous tissue, Bowman's glands will be seen in section.

Sketch as seen under the high power.

DRAWINGS FOR LESSON XXVIII.

DRAWINGS FOR LESSON XXVIII.

LESSON XXIX.*

TEETH.

(a) TOOTH.

By means of a fine saw a longitudinal section is cut from a tooth (incisors or canines are best). Grind this on an emery-wheel as thin as you can, then between two hones until it becomes quite transparent. Care should be taken to grind it evenly, while doing so keep the hones well moistened with water. Wash thoroughly first in water, then in alcohol; then place the section between filter-paper until perfectly dry. Sections will be mounted for you in hard balsam. The preparation is first to be studied under a simple lens, observing the shape and size of the pulp cavity, the relative proportion of the dentine, cement and enamel. The structure of these parts is then to be studied under the low power. In the enamel note the *enamel prisms*. In the dentine the *dentinal tubules* radiating from the pulp cavity, and in its peripheral portion the interglobular spaces. The cement shows the structure of bone, very seldom, however, showing Haversian canals.

Sketch the tooth as seen under the simple magnifier.

(b) SECTION OF TOOTH, IN SITU.

The anterior portion of the lower jaw of a dog was hardened in HNO_3 , a 10% solution, for 24 hours, in Müller's fluid for several weeks; was then decalcified in a solution composed of equal parts of a 10% HNO_3 and a 1% HCl . The decalcified tissue was embedded in celloidin, sectioned, and sections stained in hæmatoxylin and eosin,

*The following lessons are especially arranged for the students in the Dental Department.

and are now in oil of bergamot, mount in balsam. The preparation shows the tooth in longitudinal, the jaw in cross-section, and is given to show the attachment of the root of the tooth to the wall of the alveolus, by means of the dental periosteum. The different parts of the tooth will be recognized. The pulp is composed of a loose connective tissue, in it a number of small vessels may be observed in section; on the surface of the pulp a layer of odontoblasts is seen.

Sketch the preparation as seen under low power.

(c) **CROSS-SECTION OF DECALCIFIED TOOTH.**

A human tooth was hardened and decalcified in Pere-ney's fluid, embedded in celloidin, cross-sectioned, double stained in hæmatoxylin and acid fuchsin. Sections are now in oil of bergamot, mount in balsam.

The preparation is given to demonstrate the structure of the pulp. Study under high power. Observe the loose connective tissue, consisting of a few fibrils and branched connective tissue cells. A layer of odontoblasts bounds the pulp, these cells have a body somewhat columnar in shape, from this two kinds of branches are given off; pulpal processes communicating with the branched cells of the pulp and tubular processes, which enter the dental tubules.

Sketch as seen under the high power, a portion of the pulp with odontoblasts and a segment of the adjacent dentine.

DRAWINGS FOR LESSON XXIX.

DRAWINGS FOR LESSON XXIX.

LESSON XXX.

DEVELOPING TEETH.

A number of preparations, showing several stages in the development of a mammalian tooth will be given. The difficulty to obtain material showing DEFINITE stages, prevents a full description of the sections to be studied in this lesson. The student will need to rely on the notes taken at the time.

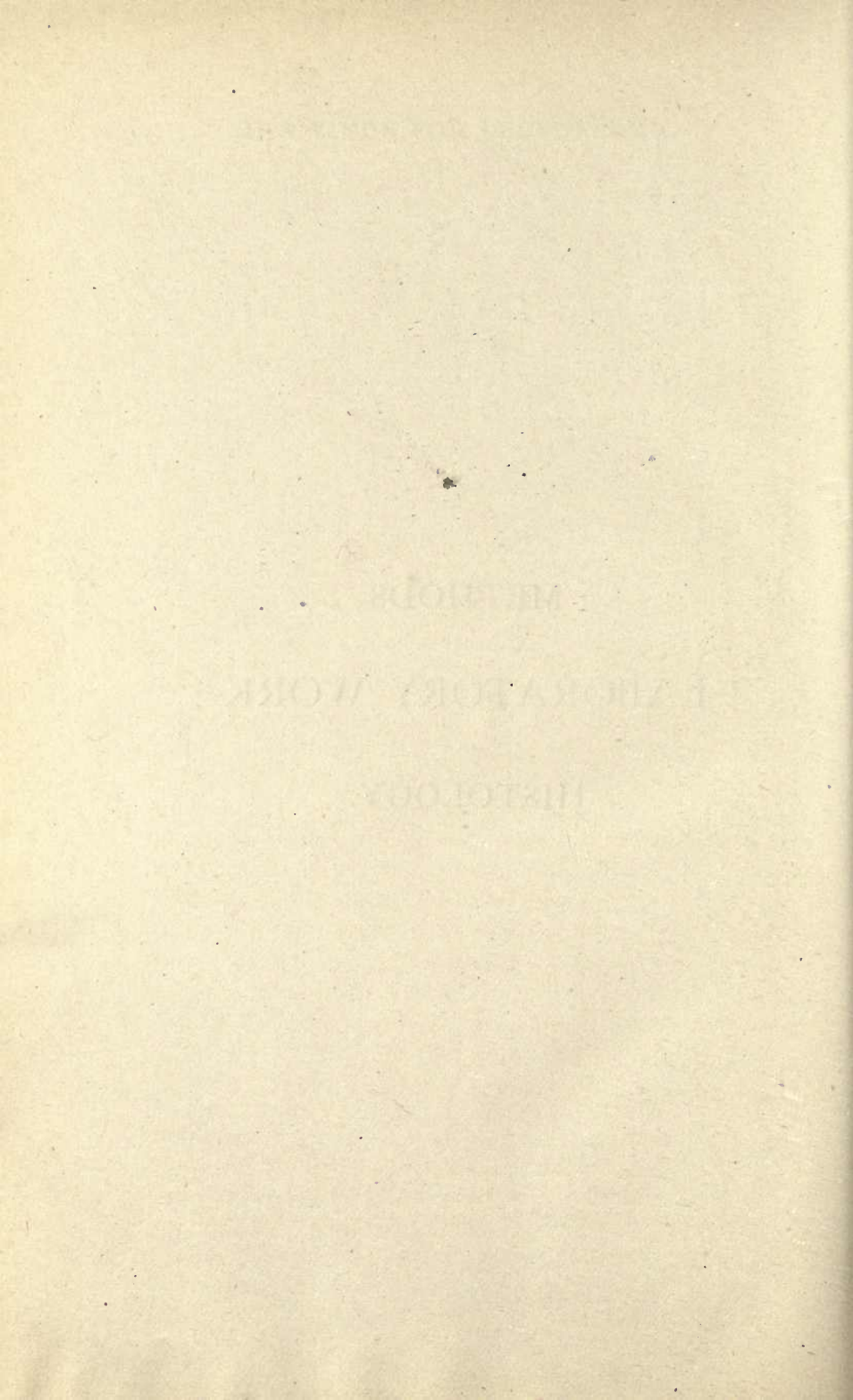
NOTES.

DRAWINGS FOR LESSON XXX.

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METHODS
FOR
LABORATORY WORK
IN
HISTOLOGY.



METHODS FOR MACERATING.

RANVIER'S ALCOHOL.

Place small pieces of the tissue to be macerated in 33% alcohol, in which they remain from 12 to 24 hours, then transfer them to a 0.25% osmic acid solution for two to four hours. They can now be teased.

This method is very useful for macerating epithelial tissue, for instance, the cells lining the intestinal canal or the trachea, for isolating the cells of the liver, etc.

CAUSTIC POTASH.

Make a 30% solution of KOH in water, small pieces of the tissue are placed in this solution for about 15 minutes. The maceration is then interrupted by transferring the tissue to a saturated, watery solution of acetate of potash, (it takes about 60 parts of the acetate of potash to saturate 40 parts of water), to which a few drops of glacial acetic acid have been added, (the author adds five to six drops to 25 cc. of the saturated solution). In about 30 minutes the tissue is ready for teasing; it can, however, be kept a long time, several months, in the acetate of potash.

This method is used in macerating non-striped and heart muscle, also epithelial cells.

HYDROCHLORIC ACID.

A 30% watery solution is used, in it the tissues remain for 12 to 15 hours, are then washed in flowing water for half an hour, they may then be teased. HCl is especially useful for isolating the tubules of the kidney. The macerated pieces of kidney may be placed in a test tube contain-

ing a 2 % watery solution of bismark brown, in this they are shaken for 10 to 15 minutes; they are at the same time stained and isolated. Allow the test tube to stand for a few moments, the isolated tubules can then be pipetted from the bottom.

NITRIC ACID. [Gage.]

A 30% watery solution is used, small pieces of the tissue remain in the fluid about 24 hours, and are then thoroughly washed in water.

Striped muscle fibres are well macerated by this method.

SULPHURIC ACID. [M. Schultz and Ranvier.]

Place the tissue to be macerated on the slide, add a few drops of the strong sulphuric acid, and cover with the cover-glass. In a few moments the cells can be separated by gently tapping or pressing with a needle the top of the cover-glass.

Useful for macerating hair, nail and horny epidermis.

SCHULTZE'S MIXTURE.

One or two grams of crystals of chlorate of potash are mixed with a little HNO_3 , only enough acid is used to make a thick paste. In this the tissue to be macerated is embedded for two or three hours. At the end of which time the tissue may be removed to a slide and teased. If it does not tease easily, embed again in the paste, repeating at intervals of about thirty minutes, until it teases readily.

This method brings out nicely the branched muscle fibres. A frog's tongue may be used.

OSMIC ACID.

A 1.0% watery solution of osmic acid is very useful in macerating retina, olfactory membrane, etc. The tissues remain in the fluid from 24 to 48 hours, and may then be teased.

ACETIC AND CHROMIC ACID. [Arnold.]

Tissue is placed for 10 to 15 minutes in a 1 % solution of acetic acid, for 24 hours in 0.01% chromic acid.

This method is useful for macerating spinal ganglia.

After washing well in flowing water the teased elements may be stained in a 1 % solution of methylen-blue.

It is best to mount teased preparations in gum glycerin, this may be made after the following formula, as suggested by Farrant.

Glycerin,	50 c. c.
Water,	50 c. c.
Gum Arabic (powder),	50 grms.
Arsenous acid,	2 grms.

Or in glycerin gelatin (Fol.):

Water,	42 c. c.
Glycerin,	38 c. c.
Gelatin,	7 grms.
Carbolic acid,	1 gm.

METHODS FOR HARDENING.

ALCOHOL. [Strong].

When possible to cut tissue into small pieces, about one quarter to one-half inch cube, it is best to place them at once into 95% alcohol, here they remain for four to five days, are then transferred to absolute alcohol for 24 to 48 hours. It is always well to place a layer of absorbent cotton into the hardening jar to prevent the tissues from resting on the bottom. The fluid can then more readily touch all surfaces of the tissue to be hardened. Gland tissue, such as salivary glands, and pancreas, the intestinal canal are well hardened by this method. Kahlden states that this method is especially useful when the tissues are to be examined for bacteria.

ALCOHOL. [Graded].

If it is not possible or advisable to cut the tissue into small pieces, it is better to place them in alcohol of about 60% in which they remain for twelve hours; then into alcohol of 75 per cent. for 24 hours; finally into alcohol of 95 per cent. for eight to sixteen days, at the end of which time they will be ready for cutting. When used in this way, alcohol penetrates more easily into the large pieces.

MÜLLER'S FLUID. [Heinrich Müller].

The "fluid" is made after the following formula:

Potassium bichromate	2.5 parts.
Sodium sulphate	1 part.
Water	100 parts.

The potassium bichromate and sodium sulphate are to be ground in a mortar. They dissolve quickly if the water is heated. A large quantity of the fluid may be kept on hand as it does not deteriorate by standing. Müller's fluid is especially to be recommended when large pieces are to be hardened, but it must be remembered the hardening takes place very slowly. Pieces of about an inch cube harden in two to four weeks, of two inches cube 1-2 months and larger masses proportionately longer—a human brain for instance needs to be in the fluid from 6 to 8 months. *Always use a large quantity of the fluid and change whenever it becomes turbid.* Weigert recommends that the jar containing the tissue to be hardened, especially if it be the central nervous system, be kept in a warm oven at a temperature of 30°-40°C, a spinal cord may in this way be hardened in one to two weeks. After hardening in Müller's fluid the tissues need to be well washed in flowing water for several hours, are then placed into 75 per cent. alcohol for two to three days and into 95 per cent. for four to six days; they are now ready for cutting. Hans Virchow⁽¹⁾ recommends that the preparation be placed from the Müller's fluid into 96 per cent. alcohol, the tissues must however be kept in the dark.

This method is especially useful for hardening the central nervous system, and when necessary to harden large masses, entire organs, tumors, etc. It is not used to advantage when the finer structure of cells is desired.

BICHLORIDE OF MERCURY. [Heidenhain].

A 0.5 per cent. sodium chloride solution is saturated with mercuric chloride; to do this bring seven to eight grams of the bichloride of mercury into 100 c. c. of the salt solution and boil, the solution becomes supersaturated, and on cooling some of the bichloride of mercury is deposited on the bottom of the bottle in the form of needle-shaped crystals, and a clear saturated solution is obtained. This solu-

(1) Quoted by Rawitz—Leitfaden Histologischer Untersuchungen.

tion does not penetrate well, so that the pieces to be hardened must not be larger than about $\frac{1}{4}$ inch cube, they remain in the fluid from two to four hours, and are then, either thoroughly washed in flowing water (several hours) and hardened in graded alcohol, remaining in each of the solutions (60 per cent.—seventy-five per cent.—95 per cent. alcohol) for 24 hours; or they are at once placed into 70 per cent. alcohol, in which they remain for 24 hours, changing the alcohol several times, and then placed into 95 per cent. alcohol. A few crystals of the iodide of potassium are with advantage added to the 70 per cent. alcohol, as bichloride of mercury is readily soluble in solutions of this salt.

It is very necessary that the bichloride of mercury be well washed out of the tissues, or the student will be annoyed with the crystals of this salt making their appearance in the sections.

This solution is one of the best hardening fluids the histologist possesses, hardens rapidly and well if the pieces are small. It may be warmly recommended for hardening small bits of tissue that have been removed from growths for diagnostic purposes. The mucous membrane of intestinal canal, gland and muscle tissue are well hardened by it.

NITRIC ACID. [Benda].

Benda recommends a 10 per cent. solution, in this the tissues remain from 24 to 48 hours, are then transferred to Müller's fluid for one to two weeks, thoroughly washed in flowing water for several hours, the hardening is completed in "graded alcohol." This method gives good results when it is desired to harden an entire eye, the HNO_3 fixes the tissues and it can then be placed into Müller's fluid without collapsing.

Skin, scalp are also well hardened after this method.

CHROMIC ACID.

The method here given is the one recommended by Prof. Gibbes.⁽¹⁾ A one-sixth per cent. watery solution of

(1) Practical Histology and Pathology. Third edition, page 16.

chromic acid is made, of this *two parts* are mixed with *one part* of 95 per cent. alcohol, the mixture is to be well stirred. The pieces of tissue must be small, about $\frac{1}{2}$ inch cube. Change the fluid at the end of the first, third and fifth day; they are hardened in eight to twelve days. Wash well in flowing water for several hours, the hardening is completed in "graded alcohol."

PICRIC ACID.

A saturated watery solution is kept on hand; filter before using. The tissue needs to be cut into small blocks, they remain in the fluid for one to three days, are then rinsed in water, and placed in 80 per cent. alcohol, which must be changed as often as it becomes yellow; as soon as none or very little of the acid is given off, place in 95 per cent. alcohol. Peripheral nerves, vessels, elastic cartilage, and fibro cartilage are well hardened in this way. Fœtal bones are decalcified.

FLEMING'S SOLUTION. [Chromic, osmic, acetic acid solution.]

One of the best of hardening solutions is a mixture of chromic, osmic, and acetic acid in the following proportions:

Osmic acid (2% watery sol.)	4 parts.
Chromic acid (1% watery sol.)	15 parts.
Glacial acetic acid	1 part.

The solution may be kept on hand in a well stoppered bottle. Tissues must be in small pieces, one dimension of which, at least, ought not to be more than one-twelfth or one-eighth of an inch. The chromic acid might penetrate larger pieces, the osmic not. The tissues remain in the solution about 24 hours, are then thoroughly washed in flowing water, the hardening is completed in "graded alcohol." This solution is largely used in hardening tissues for cell division, and for bringing out the finer details in the structure of the protoplasm and nucleus. Unless tissues are well washed it is often hard to stain them.

HERMANN'S SOLUTION. [Platinum chloride, osmic, acetic acid solution.]

Hermann uses in place of the chromic acid in Fleming's solution a 1 % watery solution of platinum chloride, the formula reading as follows:

Platinum chloride (1 % watery sol.)	15 parts.
Osmic acid (2% watery sol.)	2-4 parts.
Glacial acetic acid	1 part.

Pieces need to be small, they remain in the solution for 24 to 48 hours—washed in water—hardened in “graded alcohol.” Hermann's fluid is used with good results in hardening for karyokinesis, spermatogenesis, etc.

Bichloride of mercury may with advantage be added to either Fleming's or Hermann's solution, it seemed to Dr. Benda and the author that the accessory nucleus was better fixed when this was done. The following formulas may be given:

Bichloride of mercury (saturated water sol.)	3 parts.
Platinum chloride (1 % watery sol.)	3 parts.
Osmic acid (1 % watery sol.)	3 parts.
Acetic acid (Glacial)	1 part.

To be used as Hermann's fluid.

Bichloride of mercury (saturated watery sol.)	3 parts.
Chromic acid (1 % watery sol.)	4 parts.
Osmic acid (2 % watery sol.)	2 parts.
Glacial acetic acid (33 % watery sol.)	1 part.

To be used as Fleming's solution.

METHODS FOR DECALCIFYING TISSUES.

Kahlden* gives the following general directions to be observed when decalcifying:

1) The tissues must first be well hardened in alcohol or Müller's fluid.

2) A large quantity of the decalcifying fluid needs to be used, and changed frequently.

3) After decalcification the tissues must be thoroughly washed in flowing water for several days.

4) They are again hardened in "graded alcohol," after which they are ready for cutting.

NITRIC AND HYDROCHLORIC ACID.

Use the following proportions:

Nitric acid (10 % watery solution)	5 parts.
Hydrochloric acid (1 % watery solution)	5 parts.

The decalcification is quite rapid, the fluid needs to be changed every second or third day. The tissues are from time to time taken from the fluid and tested, by pushing a needle into the bone, and if it enters easily and without grating, the decalcification may be considered complete. They are then washed in flowing water and hardened in "graded alcohol."

EBNER'S DECALCIFYING FLUID.

The following formula is taken from "Behren's Tabellen":

*Technik der Histologischen Untersuchung pathologisch-anatomischer Präparate. Third edition. Page 13.

Sodium chloride	2.5 grms.
Water	100.0 c.c.
Alcohol	500.0 c.c.
Hydrochloric acid	2.5 c.c.

This solution decalcifies very slowly, but without injury to the tissues. Large quantities (10 to 20 times the bulk of the tissue) need to be used, and one or two c.c. of hydrochloric acid are daily added to the fluid until decalcification is complete. Wash very thoroughly in flowing water, and harden in "graded alcohol."

HAUG'S CHROM-OSMIUM SOLUTION.

Osmic acid (1 % watery solution)	10 c.c.
Chromic acid (1 % watery solution)	25 c.c.
Water	60 c.c.

Useful for decalcifying cochlea; acts very slowly. Wash in flowing water, and harden in alcohol.

X LEPKOWSKI'S METHOD.*

Small pieces of tooth or bone are placed in the following solution:

Chloride of gold (1 % watery solution)	6 parts.
Formic acid	3 parts.

Tissues are decalcified in two or three days. The chloride of gold is deposited in the dentinal tubules or canaliculi, so that the tissue will prove to be stained and decalcified at the same time.

*Anatomischer Anzeiger, 1892.

IMPREGNATION OF TISSUES.

SILVER NITRATE.

Used for staining the endothelial membranes lining blood-vessels and serous cavities. An albumenate of silver is formed with the intercellular cement between the endothelial cells; the silver is reduced on exposing to sunlight. A 1.0 per cent. watery solution of the silver nitrate is used. To make a preparation of an endothelial membrane, the peritoneum of a frog may be selected; the following steps are taken: make a small opening through the abdominal wall of a frog near the sternum, inject 10 to 20 c. c. of the one per cent. solution of silver nitrate; while injecting the abdomen is gently kneaded, so that the fluid may be well distributed over the abdominal cavity. In 15 to 20 minutes the abdomen is opened, the intestinal canal with the mesentery is removed, the latter (without removing from the intestines) is spread out on a "cork board," is now immersed, preparation side up, in 80 per cent. alcohol (it will be necessary to fix small lead weights to the cork to bring it under the alcohol) and placed in the sunlight; as soon as the tissue shows a brown color it is ready for study. It may be mounted in gum glycerin or balsam.

GOLD CHLORIDE METHOD.

After Golgi.

Small pieces of fresh tissue are placed into a 0.5 per cent. solution of arseneous acid, in which they remain until they become transparent (usually five to ten minutes), they are then placed into a 0.5 per cent. watery sol. of gold chloride about half an hour, in the dark. They are now placed in a one per cent. solution of arseneous acid

in which they are exposed to sunlight until the gold is reduced. Mount in gum-glycerin. Used for staining axis cylinders and nerve end organs, but especially for muscle end plates.

After Ranvier.

For staining nerve endings in epithelial tissue, cornea, etc., place a small piece of the tissue into the following solution :

Chloride of gold (1 per cent. watery sol.),	4 parts.
Formic acid,	1 part.

In this they remain for twenty four hours, in the dark. Expose to sunlight, in distilled water, to which a few drops of acetic acid have been added, until the gold is reduced.

SILVER NITRATE AND BICHROMATE OF POTASH METHOD.

[Golgi, Ramon J. Cajal and Lenhossek].

Used for staining nerve cells and their processes in the central nervous system and the periphery. The formulæ here given are taken from Lenhossek's "referat" in *Fortschritte der Medicin*, August and September, 1892.

Golgi's Slow Method.

Small pieces of brain or spinal cord are hardened in a 2 per cent. solution of bichromate of potassium from twenty to thirty days; are then, without washing, transferred to a 0.5 per cent. solution of silver nitrate, in which they remain from twenty-four to forty-eight hours; or in place of the silver, a 0.5 per cent. solution of bichloride of mercury may be used, in this they remain from two to four weeks.

The Mixed Golgi Method.

The tissues to be hardened and stained are placed in a large quantity (20 to 30 times the bulk of the tissues used), of the following solution :

Bichromate of potassium (1 per cent. watery sol.),	8 parts.
Osmic acid	(1 per cent. watery sol.), 1 part.

In this this they remain four or five days; are then transferred to 0.75 per cent. solution of silver nitrate twenty-four to thirty hours.

Rapid Method. [Ramon J. Cajal].

The following solution is now largely used:

Bichromate of potassium (3 to 5 per cent. sol.), 4 parts.
Osmic acid, . . . (1 per cent. watery sol), 1 part.

If it is desired to stain neuroglia cells, allow the tissues to remain in the solution two or three days, if nerve cells, three to five days; if nerve fibres and collateral branches, five to seven days. They are then transferred to a one per cent. watery solution of silver nitrate, in which they remain from twenty-four to thirty-six hours. Lenhossek adds one drop of formic acid to 200 c. c. of the silver solution.

These methods give the best results when embryonic tissues or tissues taken from newly born or young animals are employed.

Often when the methods above noted do not give good results at the first trial, they may with success be repeated on the same tissue. If after the tissues have been two or three days in the silver nitrate, the trial sections (free-hand sections cut from the blocks, mounted and examined in 95 per cent. alcohol) show no staining, they may again be placed in the bichromate of potassium solution for several days, and then again into silver. Good results follow a second and even the third trial.

Sections are cut into 95 per cent. alcohol; they may be made either "free-hand" or after embedding in celloidin, with the microtome. From the alcohol, sections are placed for 15 minutes into creosote, then washed from three to five minutes in the oil of turpentine, out of which they are taken and arranged on the slide. The excess of oil of turpentine is removed with filter paper, and sections are covered with balsam. The slide is now *carefully* heated over an alcohol flame, until the balsam becomes so

thick that on cooling, it at once hardens (three to five minutes of careful heating are required, allow no bubbles to form); while the balsam is yet warm cover with a cover-glass which has been passed through the flame several times; after cooling the preparation is ready for study. It will be found when mounted in this way Golgi preparations *do not fade*.

METHODS FOR EMBEDDING AND CUTTING SECTIONS.

Free-hand cutting. It is usually necessary to embrace the tissues to be cut with some material, which gives no resistance to, and does not injure the knife, at the same time is firm enough to give support. Pathologists have for a long time used small pieces of amyloid liver which had been hardened in alcohol or Müller's fluid. If these can not be obtained, pig's or calf's liver will answer. Elder-pith is also used. The little blocks of liver tissue, or the elder pith rods, are divided into two parts, between which the tissue to be cut is embraced. It can now be firmly held between the thumb and the index finger of the left hand. A razor, which is flat on one side (the under while in use), is employed. Its upper surface is well covered with 80 per cent. alcohol. Try to make the section with one continuous cut, resting the blade of the knife on the index finger. Sections which answer for purposes of orientation can easily be made in this way. They are, however, not to be compared with the ones that can be cut with the microtome when the tissues are properly embedded.

A great many methods for embedding tissues are in use, the principal involved is in all the same. In the one case the tissues are permeated with substances that are fluid when warm and become hard enough to cut on cooling; others need to be frozen to give them this consistency; in others again, the embedding mass hardens, on the evaporation of a solvent which was used to bring it to a fluid state. The methods for embedding in celloidin, in

paraffin, and in a solution of gum arabic (for the freezing microtome) are here given. It is deemed beyond the scope of these notes to go into the methods for *cutting sections*, they will be taught in the laboratory.

EMBEDDING FOR CUTTING ON THE FREEZING MICROTOME.

To prepare the tissues for cutting on the freezing microtome, the following steps are taken: remove the alcohol from the hardened tissues by allowing them to remain in water about eight hours, they are then transferred to a solution of gum arabic, (solution should be about as thick as syrup, and is made by dissolving the gum arabic in hot water, and straining through a cloth; allow to cool before using.) in about six hours they are permeated with the gum arabic and are ready for freezing. Sections need to be cut into warm distilled water, this removes the gum arabic. Before staining or placing in alcohol they must again be washed in distilled water.

CELLOIDIN OR COLLODIUM.*

A *stock solution* of celloidin or collodium is kept on hand. This is made by adding celloidin or collodium to a mixture of equal parts of absolute alcohol and ether until a thick solution is obtained. The stock solution needs to be kept in a "well stoppered" bottle, as it thickens on the evaporation of the alcohol and ether. The steps for embedding tissues are the following:

I. From the 95 per cent. alcohol the tissues are placed into absolute for 24 to 48 hours.

II. Into a mixture of *equal parts* of absolute alcohol and ether from one to two days.

III. Into a *thin solution of celloidin*, consisting of one part of the "stock solution" and two parts of the

*The methods for embedding in celloidin and collodium are the same. the results seem to the author equally good; collodium is cheaper. The steps here given for the one will answer for the other.

alcohol and ether mixture (equal parts); in this they remain, according to the size of the pieces embedded, from two or three days to as many weeks.

IV. Into the "stock solution" for an equal length of time. The tissues are ready for further treatment as soon as they are thoroughly permeated with the celloidin; no definite time can be fixed for this. Loose tissues, such as lung, are permeated in three or four days; skin in two or three weeks; brain or spinal cord, especially if the pieces are large, in three to six weeks.

As soon as permeation seems complete, some of the "stock solution" is poured into a flat glass dish (enough to cover well the tissues to be embedded), into this the tissues are brought, and if several pieces are to be embedded, they are to be so arranged that a small area of celloidin (one-fourth of an inch wide) surrounds each piece. The dish is now covered with a glass plate and placed under a bell-jar. The alcohol and ether evaporate very slowly, on doing so the celloidin hardens. In case the glass plate fits tightly, push it to one side, thus leaving a small space uncovered. Several days are required for the celloidin to become hard. As soon as the mass is so *firm* that it can not be indented with the finger, it is removed from the glass dish, and can now be "trimmed" into a square block containing the tissue; or into several, if a number of pieces were embedded. These celloidin blocks are now to be fixed to small cylinders of hard wood. The diameter of the wooden blocks should be a little larger than the celloidin block, and about one-half an inch long. This is done by immersing one end of the wooden cylinder in the "stock solution," a layer of celloidin is in this way spread over this end; the block of celloidin is now pressed against this layer of celloidin, placed under a bell-jar for about an hour, and it will be found to adhere quite firmly.

On cutting sections of a tissue embedded in celloidin, the knife should be moistened with 80 per cent. alcohol; sections are cut into weak alcohol or distilled water. The

celloidin blocks, even after they are fixed to the wooden cylinders, may be kept for a long time in 70 per cent. alcohol.

The celloidin method is now very largely used; is especially useful when desired to cut sections of the central nervous system; of an entire eye; of tissues or organs containing much fibrous tissue. It may be used whenever not very thin sections (less than 10 μ . to 15 μ .) are required. The celloidin or collodium need not be removed before staining or mounting; and unless the aniline dyes are used for coloring, very little stain is taken by the celloidin. The sections need to be *cleared* in oil of bergamot, as the oil of cloves dissolves the celloidin. The following method for staining and mounting celloidin sections *in series*, is recommended by Weigert: *

I. A clean glass plate is covered with a thin layer of a solution of collodium; this is to be spread out as evenly as possible over the entire surface. The plate is now placed on edge, the collodium allowed to dry; care being taken to keep the dust from it.

II. A section is placed on a strip of "closet paper," near one end. Succeeding sections, as soon as cut, are placed to the right of it. They are removed from the knife to the strip of paper, by holding the paper extended under the knife, and slipping the section onto it. To keep the sections from drying, the strip of paper is, after the removal of every section, placed in a flat dish, in which several layers of filter paper, have been spread out and thoroughly saturated with 80 per cent. alcohol. On each strip of paper is arranged only one row of sections; the strips are kept in the dish in the order used.

III. As soon as a number of strips have been covered with sections, they are arranged, sections downward, on the layer of collodium above described, and gently pressed to it. The strips of the paper can now be removed, the sections adhering to the layer of collodium. Several

*Taken from Rawitz *Leitfaden der histologischen Untersuchungen*.
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layers of filter paper are now pressed over the sections, in this way removing as much of the alcohol as possible.

IV. Before the sections have time to dry a layer of the collodium solution is poured over them, equally distributed, and allowed to dry. As soon as dry the plate may be placed into 80 per cent. alcohol, where it may be kept, or into the stain, the layer of collodium (containing the sections) then separates from the glass plate, and can be treated as a single section.

PARAFFIN EMBEDDING.

The tissues are permeated with melted paraffin; on cooling, the paraffin congeals and can then be cut. The steps for embedding are the following:

1). From 95 per cent. alcohol the tissues are transferred to absolute, here they remain from 12 to 24 hours.

2). They are then placed into a substance which at the same time mixes freely with absolute alcohol and is a solvent for paraffin; turpentine, chloroform, oil of origanum, and many other substances are in use, the tissues remaining in any one of the substances named from two to six hours.

3). They are then placed in melted *soft paraffin* in which they remain from one to three hours. The paraffin known as "soft" has a melting point of about 43° to 45° C. It is kept in a fluid state in a water bath, the temperature of which can be regulated; it *should not exceed* 52° C.

4). From the "soft paraffin," the tissues are transferred to melted *hard paraffin*, this is usually a mixture of equal parts of "soft" and a paraffin with a melting point of 55° C., and answers very well for ordinary room or laboratory temperature. During the summer months, it may be necessary to use two parts of the paraffin with 55° C. melting point, and only one of the "soft," and in very warm weather even less of the "soft." In the hard paraffin, the tissues remain from two to six hours. It is essential that the paraffin while in use for embedding

should be at a constant temperature; this can easily be done when a water bath with temperature regulator, such as is found in most laboratories, is at hand. A very simple apparatus, and one that meets the requirements quite well, is shown in Fig. I. It consists of a tripod (F); a copper

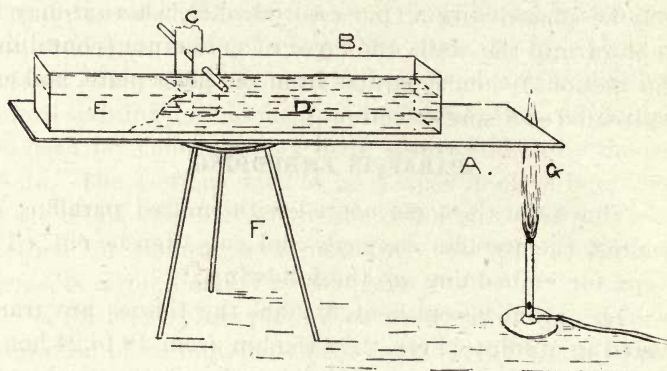


Fig. 1. Simple paraffin bath; (A) copper plate; (B) paraffin tray; (C) loop of filter paper; (D) area of melted paraffin; (E) area of unmelted paraffin; (F) tripod; (G) plane.

plate (A) about fifteen inches long, five inches wide and one-eighth to three-sixteenths of an inch thick; two tin trays (B) (only one is shown in the diagram), these are ten inches long, two wide, and three deep. They are partly filled with paraffin; the one with soft, the other with the hard. If an alcohol lamp or a Bunsen burner be so placed that the end of the flame (G) touches one end of the copper plate, as shown in diagram, and the trays containing the hard and soft paraffin be placed on the copper plate toward the end away from the flame, it will be found that after a short time, fifteen to thirty minutes, the paraffin in the end of the trays near the flame will be melted while in the other end it is yet hard, by reason of the fact that the flame end of the copper plate has a higher temperature than the opposite end. With a little patience the trays may be so adjusted, by moving them toward or away from the flame, that about half of the paraffin will be melted, the rest not. As shown in diagram (D) represents

the area of melted paraffin, (E) of the unmelted. It of course stands to reason, that if the two trays be placed side by side on the copper plate, the one containing the hard paraffin needs to be nearer the flame than the one with the soft, to obtain in each an area of melted and unmelted paraffin. In either tray, the area of unmelted paraffin acts as a thermometer, the adjoining melted paraffin must have a temperature, which when expressed in degrees, is about the melting point of the paraffin in question; about 45° C. for the soft, 50° C. for the hard. It is not advisable to allow the tissues to rest on the bottom of the tray. A loop can easily be made with a strip of filter paper about two inches wide, this is supported from a wire or glass rod, and allowed to hang in the area of melted, near the edge of the unmelted paraffin, (C) in the figure. The tissues are placed on the filter paper. When the tissues are thoroughly permeated with the hard paraffin, a rectangular trough is made with two metallic L's, resting on a glass plate, and filled with hard paraffin. Into this the tissue is placed, by means of a pair of small forceps, which before using are warmed over an alcohol flame. The piece of tissue is placed in one end, and so arranged, that the plane in which it is to be cut, is at right angles to the long axis of the trough. The paraffin is now allowed to cool, and as soon as a film forms over it, the trough is placed in cold water; this quickly congeals the paraffin. The metallic L's are removed; the paraffin block can now be taken from the glass plate and is ready for cutting. The knife used for cutting paraffin sections must be dry, and if perfectly embedded very thin sections can be cut, thinner than when embedded after any other method.

Before mounting or staining "paraffin sections," they must be fixed to the slide or cover glass. To do this, one of the following methods may be used:

Albumen Fixative. (Mayer.) The "albumen fixative" consists of equal parts of white of egg and glycerin. It is prepared by chopping the white of an egg with a pair

of scissors, then straining it through muslin or linen; it is now mixed with an equal quantity of glycerin. The glycerin and white of egg are to be thoroughly mixed by stirring with a glass rod.

A small drop of the albumen fixative is placed on a slide, and spread in a *very thin* layer with a clean glass rod, or with a dry and clean finger. The section is now placed on the albumen fixative, and pressed to the slide. If the section is *stained*, the paraffin is melted by holding the slide or cover-glass over an alcohol flame, or by placing them on a water-bath, until the paraffin begins to melt; then cover with a few drops of oil of turpentine, this dissolves the paraffin; remove excess of oil with filter paper and mount in balsam. If it is desired to stain the sections after they are fixed to the slide or cover-glass, the following steps are taken to remove the paraffin:

1. Heat quickly over the flame until the paraffin begins to melt.

2. Place the slide or cover-glass into turpentine, (chloroform or toluol may also be used) until the paraffin is dissolved.

3. Transfer to absolute alcohol for three to five minutes—this removes the turpentine.

4. Transfer to 95% alcohol for five minutes.

5. Transfer to 70% alcohol for several minutes.

6. Remove alcohol by placing slide or cover into distilled water. Sections can now be stained.

It is often quite difficult to remove folds from paraffin sections, and especially if sections are large, before fixing to the slide or cover-glass with "albumen fixative;" the author has used with success this simple method:

An evaporating dish is partly filled with distilled water; on this the sections are placed. The water is now slowly heated by holding the evaporating dish over an alcohol flame. It will be noticed that as the temperature of the water is elevated, and the paraffin in and about the sections begins to soften, the sections spread out over the

surface of the water. The water is heated until all folds are obliterated. Care should be taken not to get the water hot enough to melt the paraffin. A slide or cover-glass, on which a thin layer of the albumen fixative is spread, is now passed under one of the sections; on it the section is caught and withdrawn from the water. Allow all the water to evaporate, this usually taking 8 to 12 hours, at the end of which time the paraffin may be removed as above described. This method is very useful for mounting "serial sections."

Gaul's 50 per cent. Alcohol Method. A few drops of 50 per cent. alcohol are placed on a slide or cover-glass; on this the sections are placed. As the alcohol evaporates, the sections are fixed to the slide or cover; 12 to 24 hours are required, and it is best to place them in a warm oven at a temperature of 40° C. The paraffin is removed in the same manner as when sections are fixed with albumen fixative. The slide or cover must be very clean, even then the sections are often loosened while the paraffin is being removed. Sections from alcohol or sublimate hardened tissues seem to be most firmly fixed.

METHODS FOR INJECTING.

When it is desired to bring to prominence the relation between the blood vessels and the other elements of a tissue or an organ, it is necessary to inject the vessel. This is best done by means of substances which are fluid when warm, but harden on cooling. Gelatin, which has been colored with some dye, is usually employed. The directions for making two such injecting masses are here given. Much experience is required to inject successfully; too much space would be required to go fully into the methods used, they will be demonstrated in the *course on methods*.

CARMIN GELATIN. (Gerlach.)

The formula is taken from Behren's Tabellen.

Carmin,	10 grms.	} I.
Ammonium hydrate,	1 c. c.	
Water,	8 c. c.	
Gelatin,	12 grms.	} II.
Water,	16 grms.	

The gelatin is cut into fine pieces, placed in an evaporating dish, the water is then added; in this the pieces soak about 12 hours. The gelatin is then dissolved over a water-bath. To solution II, add solution I, which is prepared by dissolving the carmin in the ammonium hydrate and water, over a water-bath; add slowly, while constantly stirring. The mass is now alkaline, and unless neutralized would stain the bloodvessels and surrounding tissue. The neutralization is accomplished by means of glacial acetic acid, which is added drop by drop until no ammonia is detected by the sense of smell; stir-

ring well after every drop. It will also be noticed that the mass changes its color, becoming a brighter red. If the mass becomes too acid it appears granular if a drop is examined under the microscope; it is in this state not useless, but the resulting injection is never so good, as when the mass is neutral.

Before using, the mass must be strained through a piece of flannel which has been dipped in hot water. The canula, syringe, and animal must be kept warm during the injection. If the entire animal is to be injected, the canula is to be tied into the arch of the aorta, through the left ventricle; If a single organ, through its main artery.

BERLIN BLUE MASS. (Harting, as given by Rawitz.)

One part of oxalic acid is "rubbed up" in a glass mortar; to this is added one part of Berlin blue, and while constantly stirring, 12 parts of water. To an equal quantity of warm gelatin solution (made as above directed), add the Berlin blue solution, slowly and while stirring. Filter through a piece of flannel before using.

METHODS FOR STAINING.

It has long been known that, when properly hardened tissues are subjected to the action of coloring matters, certain elements of the tissues, even certain parts of the cell, show greater affinity for the stain than others. This *selective action* noticed in so many stains, warrants the place they hold in histological technic. Of the great number in use, a few of the most trustworthy, and such as can be most easily made and used, are here given.

HAEMATOXYLIN SOLUTIONS.

Böhmer's solution.—This solution is one of the best in use, and is made after the following formula :

Hæmatoxylin crystals	1 grm.	} sol. I.
Absolute alcohol	10 c. cm.	
Potash alum	10 grms.	} sol. II.
Distilled water	200 c. cm.	

The crystals of hæmatoxylin are dissolved in the absolute alcohol and kept in a well stoppered bottle for 24 hours (solution I).

The alum is dissolved in warm distilled water, allow to cool, keeping it free from dust (solution II). Add solution one to solution two, stir and keep in an open dish for about a week ; filter and the solution is ready for use.

The tissues need to be stained in section, the steps are as follows :

1. The sections come from distilled water into the stain, in which they remain from five to ten minutes.

2. Transfer to a 0.5 per cent. potash alum solution for five minutes.

*

3. Wash in distilled water—dehydrate in alcohol—clear in oil of cloves or oil of bergamot—mount in balsam.

If desired, the stain may be diluted 15 to 20 times with a 0.5 per cent. potash alum solution, in this diluted stain sections remain 12 to 24 hours, are then treated as above.

If the sections are overstained, they may be decolorized in a one per cent. solution of acetic acid until the proper *tone* is obtained; they must however, be well washed in water after such decolorization.

Ehrlich's hæmatoxylin solution.—This solution can be kept for a long time, it seems to improve with age.

Hæmatoxylin crystals	2 grms.
Absolute alcohol	20 c. cm.
Glycerin	100 c. cm.
Distilled water	100 c. cm.
Absolute alcohol	80 c. cm.
Glacial acetic acid	10 c. cm.
Potash alum to saturation.	

Mix the distilled water, the glycerin, the 80 c. cm. of absolute alcohol and the 10 c. cm. of glacial acetic acid. Dissolve the hæmatoxylin crystals in the 20 c. cm. of absolute alcohol, and add to the above solution, and shake well for several minutes.

The solution so obtained should have a reddish color and is now to be saturated with the alum; filter at the end of 24 hours.

Ehrlich's hæmatoxylin needs to mature from one to two months before it can be used. Steps for staining are as follows:

1. Sections remain in the stain from 10 to 30 minutes (they do not overstain).

2. Wash in distilled water—dehydrate—clear in oil of cloves or bergamot—mount in balsam.

Delafield's hæmatoxylin solution.—The formula is taken from Behren's Tabellen:

Hæmatoxylin crystals	4 grms.
Absolute alcohol	25 c. cm.
Ammonium alum	52 grms.
Distilled water	400 c. cm.
Glycerin	100 c. cm.
Methyl alcohol	100 c. cm.

The hæmatoxylin crystals are dissolved in the absolute alcohol, the alum in the hot water; as soon as the alum solution cools add the hæmatoxylin solution. Allow to stand in a wide vessel from three to four days, filter and add the glycerin and methyl alcohol.

This solution is very useful for staining tissue *in mass*, before using dilute three to five times with distilled water.

The pieces of tissue remain in the stain from 24 to 48 hours, are then well washed in flowing water, dehydrated in graded alcohol, embedded in paraffin or celloidin. Embryos are well stained after this method.

*Professor Gibbs' hæmatoxylin solution.**—One pound of logwood chips is mixed in a granite kettle with 50 ounces of distilled water. Bring slowly to boiling point and then allow to boil for 10 minutes; add about an ounce of potash alum and boil 10 minutes longer, constantly stirring. Allow to cool and filter at the end of 24 hours and add ten ounces of alcohol. Before using dilute with distilled water; about 10 drops of the stain are filtered into a watch-crystal full of water. Sections stain for 15 minutes, are then washed in filtered "tap-water;" dehydrated in alcohol; cleared and mounted in balsam.

Haidenhain's hæmatoxylin solution.—Tissues need to be hardened in alcohol, and stained in mass. Gland tissues are well stained after this method. Small pieces of the tissue are placed in a one per cent. watery solution of hæmatoxylin crystals, in this they remain from eight to twelve hours. They are then transferred to a one per

* Practical Pathology. Page 42.

cent. watery solution of bichromate of potash from 12 to 18 hours; in this solution the tissues become jet black. Embed in paraffin.

Weigert's hæmatoxylin solution.—Used for staining the central nervous system. Tissues need to be hardened in Müller's fluid, transferred without washing into alcohol and embedded in celloidin. The celloidin block is placed in the following solution of acetate of potash from 24 to 48 hours.

Acetate potash (saturated watery solution)	} equal parts.
Distilled water	

The block is then washed in 70 per cent. alcohol for 24 hours and may then be sectioned. (If desired the sections may first be cut and then placed in the above solution of acetate of potash for 24 hours and washed for several hours in 70 per cent. alcohol). Sections are now stained in Weigert's hæmatoxylin solution :

Hæmatoxylin crystals	1 gm.
Absolute alcohol	10 c. cm.
Lithium carbonate	1.2 grms.
Distilled water	100 c. cm.

The hæmatoxylin crystals are dissolved in the absolute alcohol, the lithium carbonate in the water—mix the two solutions. The sections remain in the stain 12 to 24 hours, are then washed in Weigert's differentiating fluid :

Borax	2 grms.
Potassium ferricyanide	2.5 grms.
Distilled water	100 c. cm.

In this solution the sections remain until the gray matter is clearly "mapped out." Wash in water—dehydrate; clear in oil of bergamot; mount in balsam. This method has been variously modified by a number of investigators, the most important of which is the modification recommended by Pal.

Pal washes the sections, after they have been stained in Weigert's hæmatoxylin solution, first in a 0.25% watery solution of permanganate of potassium, in which the sections remain for 20 to 30 minutes, or until the gray matter can be distinguished from the white, and completes the differentiation in the following solution:

Oxalic acid,	1 gm.
Sulphite potassium, (K SO ₃).	1 gm.
Distilled water,	200 c. cm.

In this the sections remain only a few minutes or until the gray matter has taken a yellowish color. Wash in water—dehydrate; clear in oil of bergamot; mount in balsam.

CARMIN.

Carmin has for many years held a prominent place among the stains used for coloring tissue. It is especially useful for staining them in bulk.

Grenacher's borax-carmin solution.

Carmin,	3 grms.
Borax,	4 grms.
Distilled water,	100 c. cm.
Alcohol, (70%)	100 c. cm.

The carmin and borax are dissolved in warm distilled water, allow to cool and add the alcohol; at the end of 48 hours, the solution is filtered, the filtrate must stand for several weeks before using. Tissues hardened in alcohol or bichloride of mercury are stained well after this method. The pieces remain in the stains from 24 to 48 hours, are then washed in an "acid alcohol wash" (6 to 8 drops of HCl to 100 c. cm. of 70% alcohol) from 6 to 24 hours, and then in 70% alcohol for two hours, dehydrated in alcohol and embedded in celloidin or paraffin.

Grenacher's alum-carmin solution.

Carmin,	1 gm.
Potash alum,	3 grms.
Distilled water,	100 c. cm.

Add the alum and carmin to the water; place over a flame and bring the water to the boiling point; allow to boil 15 minutes. As soon as the solution is cold, filter and it will be ready for use. Tissues hardened in the "chrome salts" are well stained in this solution; stain in mass. The pieces remain in the stain from 24 to 48 hours (even longer if they be large), are then washed for several hours in flowing water—dehydrated; embedded in paraffin or celloidin.

Orth's lithium carmin solution.

Carmin,	2.5 grms.
Lithium carbonate,	1.2 grms.
Distilled water,	100 c. m.

The carmin and lithium carbonate are dissolved in warm water; allow to cool and filter. Sections remain in the stain 10 to 15 minutes, are then washed in "acid alcohol" or in 70 per cent. alcohol to which a few crystals of picric acid have been added; remove acid by transferring sections to 70 per cent alcohol. Dehydrate—clear in oil of bergamot; mount in balsam.

Orth's picro-lithion-carmin solution.

To three parts of the above lithium-carmin solution add one part of a saturated watery solution of picric acid. Sections stain in 10 to 20 minutes. Wash in 70 per cent. alcohol to which a few crystals of picric acid have been added.

THE ANILINE DYES.

It would lead me far beyond the scope of these notes, to mention even very briefly, the various aniline dyes that have found their way into the histological technic. Only a very limited number and such as have proved themselves most useful are here mentioned. The tissues need to be stained in sections. They nearly all stain diffusely, and need therefore to be washed in a differentiating fluid, this is usually strong or acidulated alcohol.

A solution of *methylen blue*, *methylen grün*, *bismark brown*, *dahlia*, *acid fuchsin*, *nuigrosin*, *gentian violet*, *magdala roth*, *malachit grün* or *eosin* may be made after the following formula :

Methylen blue (or any other of the above stains)	1	gram.
Absolute alcohol,	15	c. cm.
Distilled water,	85	c. cm.

The sections remain in the stain five to thirty minutes, are then rinsed in distilled water and transferred to 95 per cent. alcohol, in which they remain until no more stain is given off from the section. Dehydrate in absolute alcohol and mount in balsam.

Fleming's Safranin solution.

Safranin,	1	gram.
Alcohol absolute,	100	c. cm.
Distilled water,	200	c. cm.

Tissues should be hardened in Fleming's or Hermann's solution. Sections remain in stain 24 to 48 hours. They are then first washed in hydrochloric or picric acid alcohol (95 per cent. alcohol, to 200 c. cm. of which one drop of hydrochloric acid or several crystals of picric acid have been added), then in absolute alcohol and mounted in balsam. Safranin stains especially well the chromatic substance of the nucleus, and is largely used for staining cells in process of division.

DOUBLE STAINING.

When certain colors are combined in a solution, or used one after the other in staining, it has been found that some elements of the tissues to be stained, are colored by one of the dyes used, while others show greater affinity for the other. This fact is made use of in combining dyes for double and treble staining.

Hæmatoxylin and eosin or acid fuchsin. Sections are stained first in a hæmatoxylin solution, that of Boehmer, Ehrlich, Delafield or Gibbes may be used. The hæmatoxylin stained sections must *go from distilled* water into the eosin or acid fuchsin solution. The eosin or acid fuchsin are used in a one per cent. solution. In this the sections remain one to three minutes; are then washed first in distilled water, then in 95 per cent. alcohol until no more of the stain (eosin or acid fuchsin) is given off. Dehydrate—clear and mount in balsam. All nuclei are stained blue, the protoplasm of cells and the connective tissue red.

Orang G. hæmatoxylin (Rawitz). The tissues must be hardened in alcohol, bichloride of mercury or picric acid. Sections are placed in a saturated watery solution of Orang G. 24 hours, washed in distilled water two to five minutes, then stained in Bøhmer's hæmatoxylin.

Safranin and licht grün, (Benda,) an aniline water solution of safranin is made after the following formula:

Safranin,	1 grm.
Aniline water,	90 c. cm.
Absolute alcohol,	10 c. cm.

Aniline water is made by saturating distilled water with aniline oil and filtering, add the alcohol and the safranin. Sections remain in the stain 24 hours, are then washed in absolute alcohol for one minute, and placed from 30 to 45 seconds in a one per cent. alcoholic solution of licht grün; wash again in absolute alcohol. Clear in oil of bergamot and mount in balsam. The licht grün removes the safranin more quickly from the protoplasm of the cells than from the chromatin of the nucleus. When the stain is properly managed the chromatin is stained red, the protoplasm green.

Eosin and Nigrosin, This method is useful for staining sections of the central nervous system. Sections are stained for one hour in a one per cent. solution of nigrosin, are then washed in distilled water for several minutes; they are then placed in a one per cent. solution of eosin in which they remain from three to five minutes.

Wash first in seventy per cent. alcohol then in 95 per cent. until no stain is given off from the section.

TREBLE STAINING.

The formula here given was first recommended by Ehrlich, and used for staining blood preparations. Biondi and Heidenhain have so modified it, that it can be used for staining sections.

Acid fuchsin (saturated watery solution), . .	20 parts.
Methylengrün (saturated watery solution), . .	50 parts.
Orang G. (saturated watery solution), . . .	100 prast.

The acid fuchsin and Orang G. are placed in a bottle and mixed, the methylengrün is added slowly, while constantly shaking. The stain should not be filtered, the required amount is pipetted from the bottle. Before using dilute forty to sixty times with distilled water. Tissues need to be hardened in alcohol or bichloride of mercury. Sections remain in the stain from twelve to twenty-four hours, are then washed in alcohol, cleared in oil of cloves and mounted in balsam.

METHODS FOR PREPARING AND STAINING FOOD PREPARATIONS.

The steps for obtaining blood preparations are the following: Have before you a piece of filter paper on which are placed a number of carefully cleaned cover-glasses, these must be very thin, "extra number one." Ehrlich washes the cover-glasses first in strong sulphuric acid, rinses in water and places them for a few moments in glacial acetic acid; they are then washed in flowing water until all the acid is removed, transferred to 95 per cent. alcohol, from which they are taken and wiped. Unless the cover-glasses are thin and clean, no good preparation can be made. The finger is pricked with a steel pen, one of the prongs of which have been broken off. From the flowing blood a very small drop is caught on the cover-glass, near its edge, and the glass quickly placed, blood side downward, on another cover-glass, care being taken to cover this second cover-glass only about half. The blood will be seen to spread out between the two covers. Quickly draw one cover-glass from the other; a thin layer of blood will in this way be spread on both slips. Ten to twenty preparations are to be made in this way and placed, blood side up, on the filter paper before you and allowed to dry. The blood preparations may be fixed by placing them in a solution, composed of equal parts of absolute alcohol and ether, in which they remain for an hour as suggested by Nikiforoff or as Ehrlich recommends, by exposing them to a temperature of 100° to 130° C. for one-half to two hours.

Ehrlich has a very simple apparatus, shown in Fig. 2, for fixing blood preparations. It consists of a copper plate

H) about 15 inches long, four wide and one eighth inch thick. The copper plate is heated at one end with an alcohol or gas flame. If then, at the end of 15 minutes a

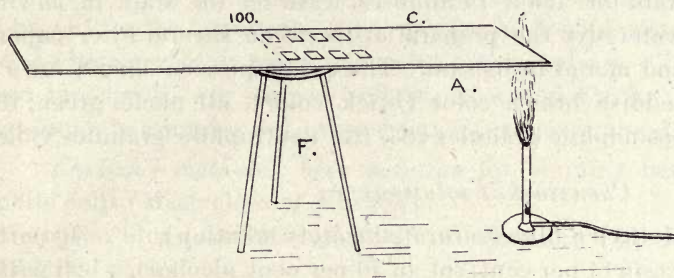


Fig. 2. plate for heating blood preparations. (A) copper plate; (C) blood preparations. F. Tripod.

glass rod which has been dipped into water be passed over the plate, beginning at the end away from the flame, a place is reached where the water begins to boil; this region of the copper plate is looked upon as having a temperature of 100° C., it is represented by a dotted line in the diagram. The blood preparations (C) are placed on the plate (blood side up) between the flame and this imaginary line, nearer the latter, and heated for a time differing with the stain used.

After the preparations are fixed they are stained in one of the following solutions :

Ehrlich's neutrophile mixture—

Orang G. (saturated aqueous solution),	120-135 c. cm.
Acid fuchsin (saturated aqueous solution),	80-165 c. cm.
Methylengrün (saturated aqueous solution),	125 c. cm.
Distilled water,	300 c. cm.
Alcohol absolute,	200 c. cm.
Glycerin,	100 c. cm.

Mix orang G. acid fuchsin, water and absolute alcohol in a bottle, add slowly and while shaking the methylen-grün. The glycerin is then added For staining in

Ehrlich's *neutrophile mixture*, blood preparations need to be fixed at a temperature from 100° to 110° C. for 15 to 30 minutes. Float the preparation on a small quantity of the stain for about 15 minutes, wash off the stain in flowing water, dry the preparation between several filter papers, and mount in balsam. The red corpuscles should have a reddish brown color (brick color), all nuclei green, the eosinophile granules red, the neutrophile granules violet.

Chenzinski's solution—

Methylen blue (saturated watery solution),	40 parts.
Eosin (1 per cent. sol. in 70 per cent. alcohol),	20 parts.
Distilled water,	30 parts.
Glycerin,	10 parts.

The eosin, distilled water and glycerin are mixed in a bottle, the methylen blue is added slowly while shaking. Preparations need to be fixed from one to one and one-half hours at a temperature of 120° C. They remain in the stain for 24 hours in the warm oven at a temperature of 40° C. Wash quickly in flowing water, dry between filter paper and mount in balsam. The red corpuscles and the eosinophile granules are stained red, all nuclei blue.

Ehrlich's triacid glycerin mixture—

Aurantia,	2 grms.
Eosin,	3 grms.
Nigrosin,	5 grms.
Glycerin,	40 c. cm.

The glycerin is divided into three parts, to each is added one of the above stains, and each needs to be ground in a mortar for several hours. The three glycerin solutions are then mixed and exposed to a temperature of 60° C. for two weeks. The stain is then ready for use and if well made amply repays all the trouble taken in making it. It will keep for a long time and should be of a syrupy

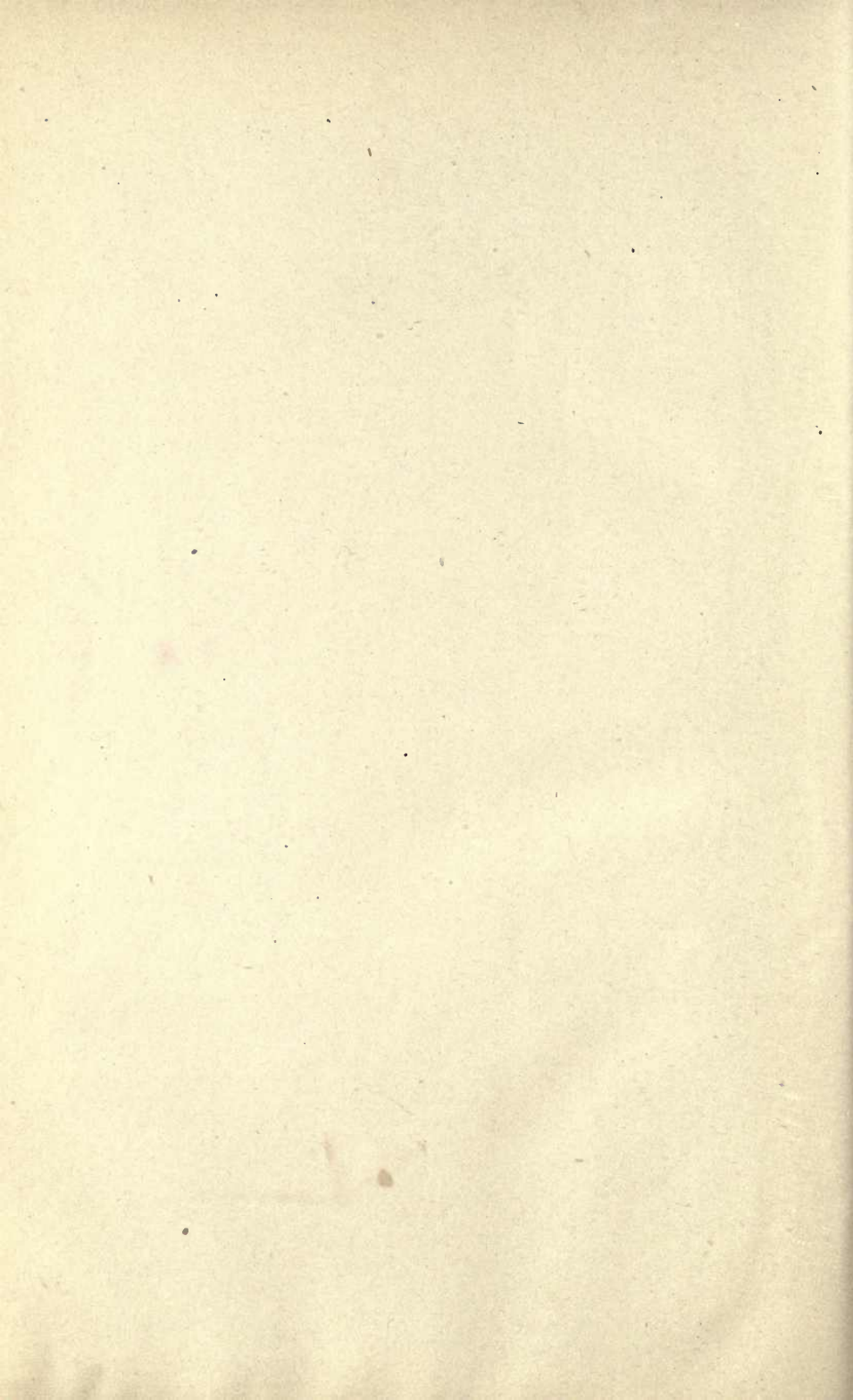
consistancy. The blood preparations need to be fixed at a temperature of 130° to 140° C. for one to two hours. A small quantity of the stain is spread out in a flat dish, on this the preparations are placed, they remain in the warm oven (40° C.) for 24 hours; are then washed in flowing water, dried between filter paper and mounted in balsam. The red blood cells are stained yellow, all nuclei black, the eosinophile granules red, other granules are not stained.

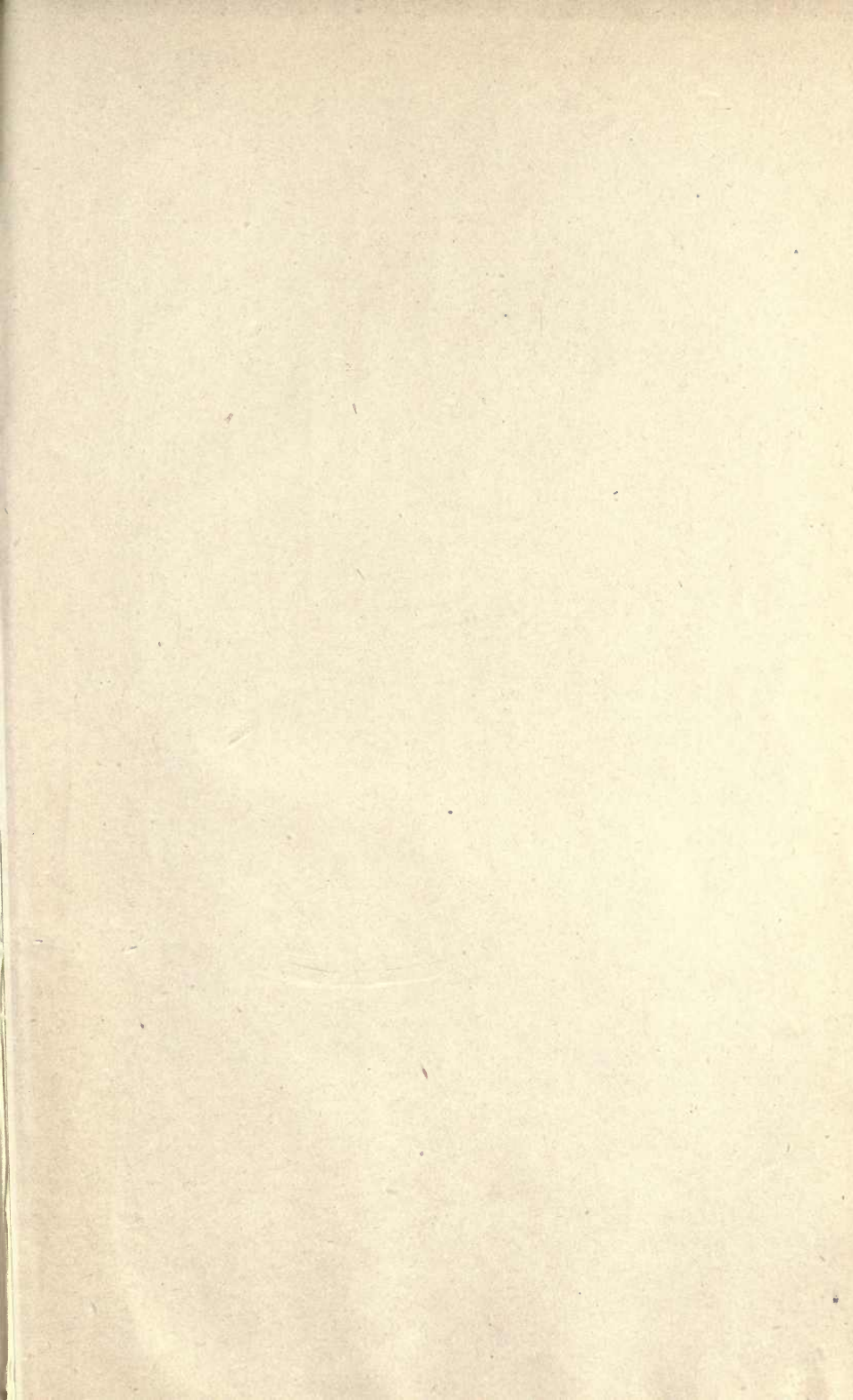
Ehrlich's methylen blue solution for staining basophile cells (Mastzellen of Ehrlich).

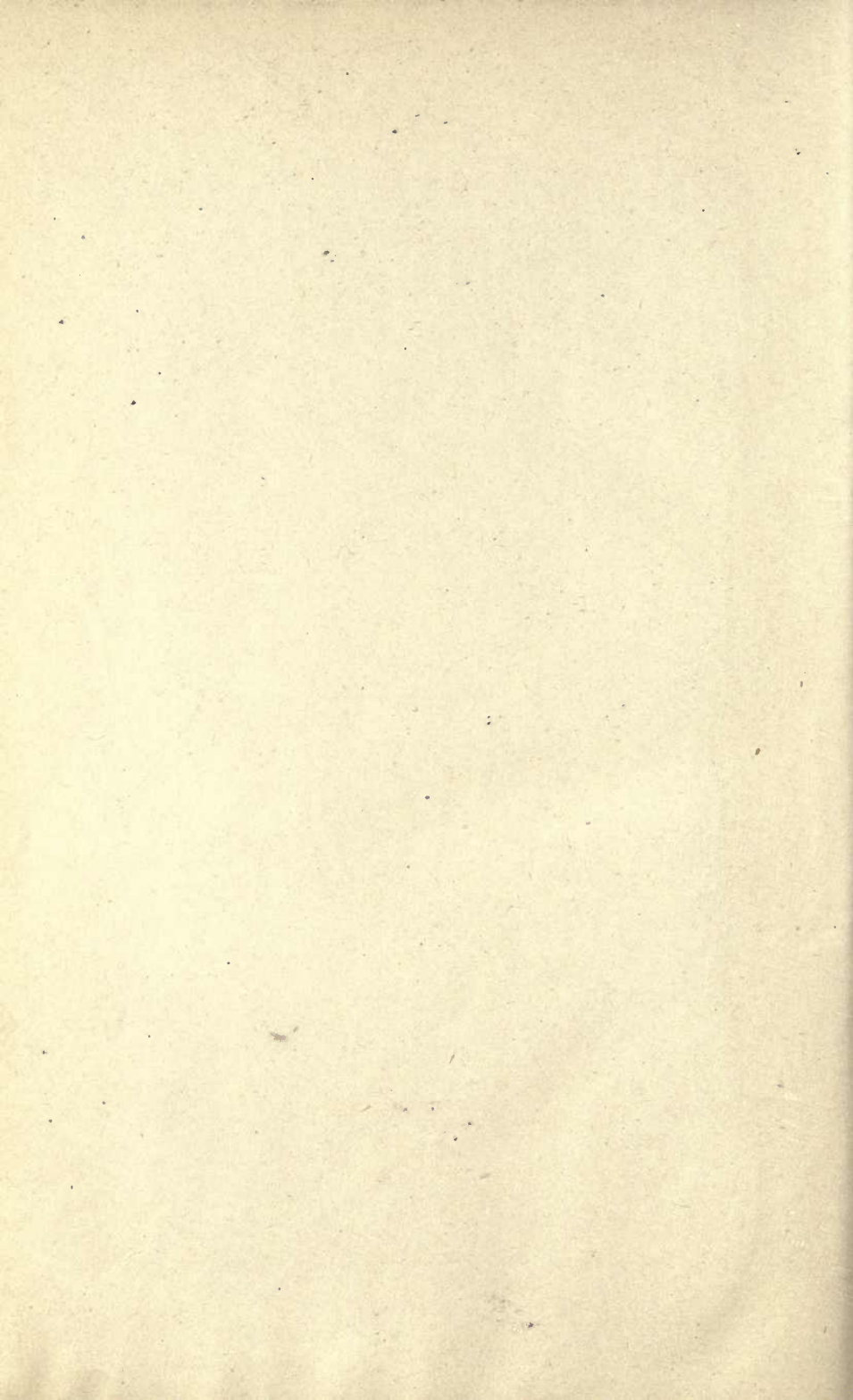
Methylen blue (saturated alcoholic solution), . . . 1 part.
Distilled water, 2 parts.

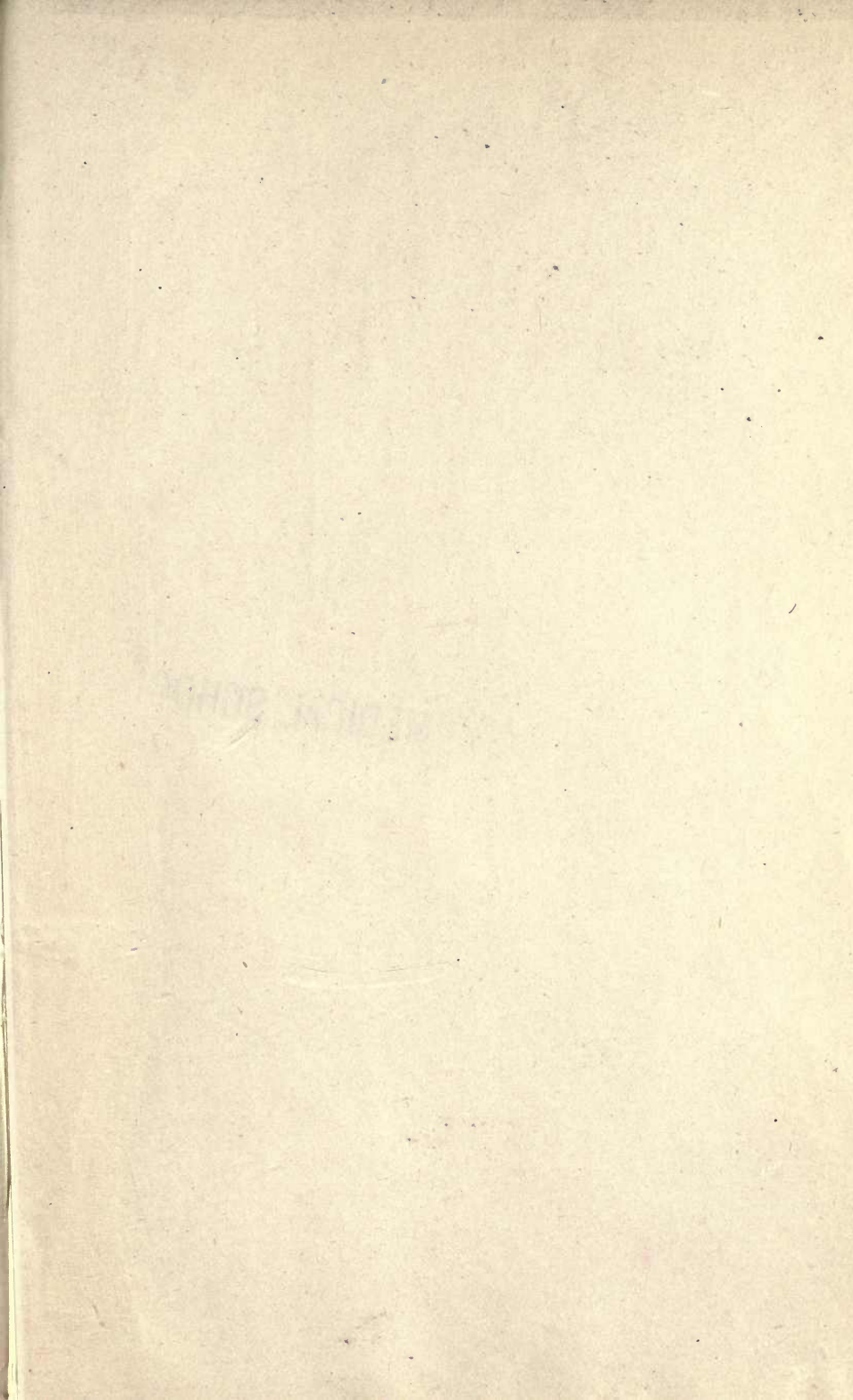
Preparations are fixed at a temperature of 110° C. for 30 minutes. Stain for 15 minutes. Wash quickly in flowing water, dry between filter paper and mount in balsam. All nuclei are stained blue, and *only* the basophile granules, which also take a blue color, are stained.

The methods here given are especially useful for studying the blood clinically, and can not be too warmly recommended.









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